SAMPLE PROCEDURE

This procedure is provided to Fisher Healthcare customers to assist with the development of laboratory procedures. This document was derived from, and was current with, the instructions for use (IFU) that accompanies the product at the time it was created. The user is instructed to consult the IFU packaged with the product to ensure currency of the procedure prior to adapting the document to routine laboratory use and periodically thereafter to ensure future IFU modifications, which might effect this procedure, are identified. Any modifications to this document are the sole responsibility of the person making the modifications.

Title: **Sure-Vue™ *Signature*** *Cryptosporidium/Giardia* Test

Procedure #:

Institution: _____

Prepared by: _____ Date: _____

Title: _____

Accepted by: _____ Date adopted: _____

Title: _____

Reviewed by: _____ Date: _____

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Discontinued by: _____ Date: _____

I. TEST NAME

Sure-Vue™ *Signature* *Cryptosporidium/Giardia* Test
CLIA Complexity: Moderate

II. INTENDED USE

The **Sure-Vue™ *Signature*** *Cryptosporidium/Giardia* Test is a rapid immunoassay for the qualitative detection of *Cryptosporidium parvum* and *Giardia lamblia* specific antigens in aqueous extracts of human fecal specimens. It is intended for in vitro diagnostic use as an aid in the detection of suspected *Cryptosporidium* or *Giardia* infections by professional laboratories.

III. SUMMARY AND EXPLANATION

Cryptosporidium and *Giardia* are recognized as two of the most frequent causes of parasitic intestinal disease. Both organisms are found throughout the world. Transmission is usually through the ingestion of contaminated food or water.

Giardiasis in humans is caused by the protozoan parasite *Giardia lamblia* (also known as *Giardia* intestinalis). The acute disease is characterized by watery diarrhea, nausea, abdominal cramps, bloating, weight loss and malabsorption lasting for several weeks. Chronic or asymptomatic infection can also occur.[1,2]

Cryptosporidiosis in humans is caused by the coccidian parasite *Cryptosporidium parvum*. Acute symptoms include watery diarrhea, abdominal cramps, loss of appetite, low grade fever, nausea and vomiting lasting for several days to over a month. Severe, persistent infections can occur in immunocompromised patients.[1] Infection may also be asymptomatic. The parasite has been implicated in several major waterborne outbreaks in the United States.[3]

Diagnosis of *Cryptosporidium* and *Giardia* infection has traditionally been done by microscopic examination of stools. More recently, the detection of *Giardia* and *Cryptosporidium* antigens in stool specimens using enzyme immunoassays has become an accepted approach to diagnosis.[4-6] The **Sure-Vue™ *Signature*** *Cryptosporidium/Giardia* Test Kit detects similar antigens using a non-enzymatic rapid immunoassay format.

IV. PRINCIPLE OF THE PROCEDURE

The **Sure-Vue™ *Signature*** *Cryptosporidium/Giardia* Test is a qualitative immunochromatographic assay that simultaneously detects and distinguishes between *Cryptosporidium* and *Giardia* antigens in aqueous extracts of patient stool specimens. The specimen, collected in a sample transport or preservative medium, is added to a tube containing a treatment buffer. A biotinylated anti-Giardia capture antibody reagent is then added, followed by a pooled suspension of colloidal dye labelled monoclonal antibodies to *Giardia* and *Cryptosporidium*. The sample is then mixed and poured into the test device that contains a capture reagent (an avidin derivative) for *Giardia*, a capture antibody for *Cryptosporidium*, and a control antibody that binds to excess colloidal dye conjugate. If *Giardia* antigen is present in the sample, a grey-black band will develop at the GIAR position in the device window. If *Cryptosporidium* antigen is present, a grey-black band will appear at the CRYP position. The appearance of a black band at the CONT position is required for the test result to be valid. It indicates that the colloidal dye conjugate is intact and that proper capillary flow has occurred.

V. KIT COMPONENTS, REAGENTS & STORAGE

Foil pouched *Cryptosporidium/Giardia* test devices consisting of: a) a membrane coated with an avidin derivative, mouse anti-*Cryptosporidium* and goat anti-mouse IgG, b) pad materials, desiccant and a plastic housing. (30 per test kit)

(2.8 mL) Sample Treatment Buffer: Buffer solution with detergent. WARNING: contains 0.1% azide.

(1.8 mL) Conjugate Reagent A: Biotinylated rabbit anti-Giardia in diluent buffer with carrier protein and detergent. WARNING: contains 0.1% azide.

(1.8 mL) Conjugate Reagent B: Colloidal dye labelled monoclonal antibodies to *Cryptosporidium/Giardia* in diluent buffer with carrier protein and detergent. WARNING: contains 0.1% azide.

(1 bag of 30) Specimen transfer pipettes.

(1 bag of 30) Specimen dilution tubes.

Store kit refrigerated 2 to 8°C (36 to 46°F) and return kit to the refrigerator promptly after each use. **DO NOT FREEZE**.

At this facility, kits are stored: _____

_____

VI. MATERIALS REQUIRED BUT NOT PROVIDED

Specimen collection and transport devices. SAF, 10% formalin, C&S, Cary-Blair, MIF.

Clock or timer.

VII. WARNINGS AND PRECAUTIONS

1. For in vitro Diagnostic Use.
2. **CAUTION**: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
3. Do not use kit beyond the printed expiration date.
4. Handle all patient samples as if they are capable of transmitting infectious disease. Dispose of them properly. Ship samples according to federal regulations regarding the transportation of Infectious agents.
5. **WARNING**: Sample Treatment Buffer, Conjugate Reagent A and Conjugate Reagent B solutions contain sodium azide. Sodium azide may react with lead or copper plumbing to form explosive metal azides. Use copious amounts of water to flush discarded solutions.
6. Do not interchange or mix components from different kit lots.

HAZARDS AND PRECAUTIONARY STATEMENTS
There are no known hazards associated with this product.

VIII. PATIENT PREPARATION & SPECIMEN COLLECTION

This facility's procedure for patient preparation is: _____

_____

This facility's procedure for sample labeling is _____

_____

Specimen Collection and Handling:
Stool specimens collected for ova and parasite examination can be used in the *Cryptosporidium/Giardia* test. The samples should be collected in clean, leak-proof plastic containers.

Samples collected in SAF, 10% formalin, MIF, Cary-Blair, C&S, or Stuart's transport media are the preferred media for specimen collection, transport and test. It is recommended that specimens collected in MIF fixative be tested before the addition of Iodine. Samples in PVA are not suitable.

Fresh (unpreserved) samples
Solid, semi-solid or liquid samples are acceptable but must be diluted 1:4 in an acceptable transport media before running the test. If transport media is not available, distilled or deionized water may be used. In cases of solid or semi-solid samples, mix the specimen using a wooden applicator or equivalent prior to dilution.

To make a 1:4 dilution:

- Remove the equivalent of 1 mL mixed specimen and place into 3 mLs of acceptable transport media.
- Mix thoroughly and proceed with test.

Cary-Blair, C&S, and **MIF** may interfere with certain confirmatory test methods. Alternative transport media for patient specimens may be recommended for confirmatory testing. As with all

test methods, product literature for product performance claims and limitations should be reviewed prior to use. **Fresh samples and specimens in Stuart's media** should be tested as soon as possible after collection, as extended storage conditions have not been validated.

Formalin and SAF preserved specimens can be stored frozen (-20ºC or -70ºC), refrigerated (2 – 8ºC) or at room temperature (20 – 30ºC) and should be tested within two months after collection.	Cary-Blair or C&S diluted samples can be stored refrigerated (2 – 8ºC) and tested within two weeks after collection or frozen (-20ºC) and tested within two months.	MIF preserved specimens can be stored frozen (-20ºC), refrigerated (2 – 8ºC) or at room temperature (20 – 30ºC) and should be tested within two months after collection.

This facility's procedure for transporting specimens is: _____

_____

This facility's procedure for rejected specimens is: _____

_____

IX. QUALITY CONTROL

Several features are incorporated into the **Sure-Vue™ *Signature*** *Cryptosporidium/Giardia* Test as routine quality checks:

1. The appearance of a control band at the CONT position verifies that a functionally intact colloidal dye conjugate has been added to the device, that the control line antibody is functionally active and that adequate capillary flow has occurred.
2. Characterized formalin preserved patient specimens may be used as routine external positive and negative controls. Characterized samples should be run as test specimens according to the procedures described below.
3. Quality Control requirements should be established in accordance with local, state and federal regulations or accreditation requirements. Minimally Cardinal Health recommends that positive and negative external controls be run with each new lot and with each new untrained operator.

QC Testing Frequency and Documentation

For this facility, external QC is run: _____

_____

Results of External QC and action(s) taken when control results are unacceptable are documented:

_____

_____

X. TEST PROCEDURE

Procedural Notes:

1. Allow kit components and specimens to equilibrate to room temperature before use. Mix liquid reagents by inverting several times before use. Return kit to the refrigerator promptly after each use.
2. Do not unpouch the test devices until ready for use.
3. Several tests may be run at the same time. Use separate dilution tubes and pipettes for each specimen.

4. To prevent possible contamination, avoid touching the dispensing tip of the Sample Treatment Buffer, Conjugate Reagent A and Conjugate Reagent B dropper bottles to the dilution tubes, pipettes, test devices or anything that has come into direct contact with patient specimens.
5. To minimize reagent waste and better control the size of drops dispensed from the reagent bottles, the following steps are suggested:
   - Store reagent bottles in upright position.
   - After removing the cap, thoroughly wipe tip with a laboratory wipe making sure all liquid is removed.
   - Hold bottles in a near vertical position for dispensing. If running multiple samples, allow air to enter the bottle before dispensing drops for the next test.
   - If liquid is observed on the tip at any time, wipe clean with a lab wipe prior to dispensing drops.
   - Do not vigorously shake or vortex reagents.
6. Do not concentrate patient specimens. When sampling, use the liquid portion of the specimen. It is recommended that particulates be allowed to settle so that the sample can be more easily pipetted.
7. All fresh or unpreserved specimens, including liquid samples, must be diluted approximately 1:4 in one of the acceptable transport media prior to use with this assay (See SPECIMEN COLLECTION AND Handling section).
8. The sequence of reagent additions to the sample tube allows the user to visually monitor the procedure: Sample Treatment Buffer is added to an empty tube; the stool specimen is visually observable; Conjugate Reagent A has a red dye incorporated in it; Conjugate Reagent B is black in color

Test Procedure:

1. Remove the test device from the pouch and place on a flat surface; place a Specimen Dilution Tube into the kit workstation holder or into a suitable test tube rack.
2. Add two drops of Sample Treatment Buffer to the Specimen Dilution Tube.
3. Use the Specimen Transfer Pipette to aspirate the aqueous patient stool specimen: squeeze the bulb of the pipette, insert the open end into the sample and release the pressure on the bulb while holding the pipette in the sample. Draw sample to the 60 µL calibration line. Transfer the contents of the pipette barrel into the Specimen Dilution Tube.

   Note: All fresh or unpreserved specimens, including liquid samples, must be diluted approximately 1:4 in one of the acceptable transport media prior to use with this assay (see SPECIMEN COLLECTION AND HANDLING section). If transport media is not available, distilled or deionized water may be used.

4. Mix/Invert Conjugate Reagent A. Add two drops of Conjugate Reagent A to the tube.
5. Mix/Invert Conjugate Reagent B. Add two drops of Conjugate Reagent B to the tube.
6. Mix the sample by manual swirling or by vortexing. Pour the entire contents of the tube into the sample well of the test device.
7. Visually read the test results at 10 min. Results are invalid after **15 minutes**.

For this facility, stool specimens and containers, used test tubes, pipettes and devices are disposed: _

_____

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In the event this test becomes inoperable, this facility's course of action for patient samples is:_____

_____

XI. INTERPRETATION OF TEST RESULTS

Note: Visible test lines in any shade of grey or black only should be read as positive. Test lines in shades of yellow or brown should not be read as positive, but considered an invalid result.

Positive for *Cryptosporidium*
The presence of grey-black bands at the CRYP and the CONT positions indicates that *Cryptosporidium* antigen has been detected. Visible test lines, in shades of grey or black only, should be read as positive. The intensity of the grey-black bands can vary from faint to strong. There is no direct correlation between the intensity of the test line with the severity of the infection.

Positive for *Giardia*
The presence of grey-black bands at the GIAR and the CONT positions indicates that *Giardia* antigen has been detected. Visible test lines, in shades of grey or black only, should be read as positive. The intensity of the grey-black bands can vary from faint to strong. There is no direct correlation between the intensity of the test line with the severity of the infection.

Negative for *Cryptosporidium*
No band is visible at the CRYP position and a visible grey-black band present at the CONT position indicates that *Cryptosporidium* antigen is absent or is below detectable levels. The GIAR position can have either a visible grey-black band or no grey-black band.

Negative for *Giardia*
No band is visible at the GIAR position and a visible grey-black band present at the CONT position indicates that *Giardia* antigen is absent or is below detectable levels. The CRYP position can have either a visible grey-black band or no grey-black band.

Invalid Results
If no band appears at the CONT position or incomplete or beaded bands appear at the CRYP or GIAR positions, the result is considered invalid. In situations where adequate flow does not occur due to excessive particulate matter in the specimen, the sample can be diluted two-fold in deionized water or in the same transport medium as it was originally collected and re-run. The test should be repeated using another device. Test lines in colors other than grey or black should be considered invalid. Repeat testing using an alternative method or different sample. If an alternative method is used, verify the preservative and the age of the sample is appropriate (refer to Specimen Collection and Handling).

XII. RESULT REPORTING

This facility's procedure for patient result reporting is: _____

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XIII. LIMITATIONS OF THE PROCEDURE

1. As with all diagnostic procedures, the results obtained with the **Sure-Vue™ *Signature*** *Cryptosporidium/Giardia* Test should be used in conjunction with other clinical information available to the physician.
2. Negative results can occur in samples containing levels of antigen below the lower limits of detection of the assay. Multiple specimens collected over several days can be tested for patients suspected of being positive for *Giardia* or *Cryptosporidium*.
3. The test is designed for use with stool samples collected in an acceptable transport media. The use of colonic washes, aspirates or other diluted sample types has not been established and could affect the performance of the assay. Stool samples contaminated by products with an oily or particulate base (eg. Barium, mineral oil, etc.) could interfere with the test and are not recommended**.**

XIV. EXPECTED VALUES

The prevalence of Cryptosporidium and Giardia is variable among different populations and geographic areas. Giardia incidence in developed countries is approximately 2-5%; Cryptosporidium incidence in Europe and North America is about 1-3%.[3] Higher prevalence rates may be present in children and in the immunosuppressed. [1-3]

XV. PERFORMANCE CHARACTERISTICS

Refer to Package Insert – **Sure-Vue™ *Signature*** *Cryptosporidium/Giardia* Test

XVI. CROSS-REACTIVITY

The *Cryptosporidium* and *Giardia* assay was run on stool specimens documented to be positive for other parasites by microscopy.

The *Giardia*-specific part of the assay showed no cross-reactivity to the following organisms:

Ascaris lumbricoides (1)
Blastocystis hominis (58)
Chilomastix mesnili (5)
Cryptosporidium parvum (121)
Cyclospora cayetanensis (1)
Dientamoeba fragilis (14)
Endolimax nana (28)
Entamoeba coli (14)
Entamoeba hartmanni (12)
Entamoeba histolytica/dispar (14)
Enterobius vermicularis (1)
Enteromonas hominis (2)
Hookworm (1)
Hymenolepsis nana (3)
Iodamoeba bütschlii (13)
Microsporidia (1)
Strongyloides sterocoralis (2)
Taenia sp. (1)
Trichomonas hominis (1)

The Cryptosporidium-specific part of the assay showed no cross-reactivity to the following organisms:

Ascaris lumbricoides (1)
Blastocystis hominis (92)
Chilomastix mesnili (6)
Cyclospora cayetanensis (1)
Dientamoeba fragilis (16)
Endolimax nana (48)
Entamoeba coli (21)
Entamoeba hartmanni (20)
Entamoeba histolyticaldispar (19)
Enteromonas hominis (2)
Giardia lamblia (132)
Hookworm (1)
Hymenolepsis nana (4)
Iodamoeba bütschlii (12)
Microsporidia (1)
Strongyloides sterocoralis (2)
Taenia sp. (2)
Trichomonas hominis (1)

The numbers in parentheses represent the number of samples tested for each organism.

XVII. REFERENCES

Refer to Package Insert. – **Sure-Vue™ *Signature*** *Cryptosporidium/Giardia* Test

XVIII. ASSISTANCE

For technical assistance please call our Technical Support Center at 1-800-332-1042.