curian®

CAMPY

A Rapid Fluorescent Immunoassay for the Detection of a *Campylobacter*-specific antigen in Stool Specimens

REF **760730**

IVD

℞ Only

For Professional Use Only

INTENDED USE

Curian Campy, for use with the Curian Analyzer, is a rapid, qualitative fluorescent immunoassay for the detection of a *Campylobacter*-specific antigen in human fecal specimens. Curian Campy is intended to detect *C. jejuni*, *C. coli*, *C. upsaliensis*, and *C. lari* in human stool from patients with signs and symptoms of gastroenteritis. The test is intended for use with unpreserved fecal specimens or preserved fecal specimens in transport media. Test results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures. Curian Campy is intended to aid in the diagnosis of *Campylobacter* infection.

SUMMARY AND EXPLANATION OF TEST

Campylobacter is a gram-negative, microaerophilic bacterium that is carried in the intestine of many wild and domestic animals, especially avian species including poultry[1]. In fact, poultry is considered a major source for *Campylobacter* infections in humans, with transmission occurring through ingestion of contaminated food and water[2, 3]. *Campylobacter* infection is referred to as campylobacteriosis, which presents with symptoms of acute watery or bloody diarrhea, abdominal pain, and fever that usually begin within 24 to 72 hours following ingestion, lasting an average of 6 days[4]. In some instances, infection can progress to life-threatening extragastrointestinal diseases[5].

Campylobacter infection is one of the most widespread infectious diseases of the last century, and evidence suggests that the incidence and prevalence of campylobacteriosis have increased in both developed and developing countries over the last decade, especially in children and the elderly[1, 4]. In the United States (from 1998 to 2008), the annual number of campylobacteriosis cases was estimated to be 845,024 cases, resulting in 8,463 hospitalizations and 76 deaths. The U.S. Food-Borne Disease Active Surveillance Network (from 1996 to 2012) reported an annual incidence of 14.3 per 100,000 population[4].

Over 90% of *Campylobacter* infections are caused by *C. jejuni*, about 5% by *C. coli* and the remainder by other *Campylobacter* species, such as *C. lari* or *C. upsaliensis*[6]. There is increasing acknowledgment of the clinical importance of *Campylobacter* species other than *C. jejuni* and *C. coli*, due to the recognition of these species as emerging human and animal pathogens[4, 5]. The conventional approach to the laboratory diagnosis of *Campylobacter* infection is through culture methods, requiring the use of specialized media and a selective incubation temperature of 42 C for several days in an artificially created microaerophilic environment in order to recover organisms from a stool specimen. Recent studies suggest that the culture methodology has its limitations, and that immunoassays are convenient and dependable alternatives for the laboratory diagnosis of *Campylobacter* infection[7, 8]. The Curian Campy assay provides rapid, reliable results for the detection of *Campylobacter* specific antigens using a fluorescent analyzer to remove subjectivity and assist in the diagnosis of *Campylobacter* infection.

BIOLOGICAL PRINCIPLES

The Curian Campy assay consists of a test strip enclosed in a plastic frame (test card), positive control reagent, and assay-specific Aioprep™ sample preparation device. Curian Campy is a lateral flow-based immunoassay for the direct detection of a *Campylobacter*-specific antigen in human stool. Curian Campy utilizes monoclonal antibodies, specific for an antigen common to *C.jejuni*, *C.coli, C. lari* and *C. upsaliensis*, as the capture and detector antibodies.

The Aioprep is pre-filled with blue tinted Sample Diluent/Negative Control and contains a filter and a sample metering device. A sample of the patient's stool specimen is transferred from the collection container to the Aioprep, using either the included transfer pipette (for preserved or unpreserved liquid/semi-solid specimens), or the assay-specific sample collection brush (for preserved or unpreserved formed/solid specimens) available separately as an accessory. When using the sample collection brush, the brush with sample is inserted through the metering device to remove excess stool and then pushed directly into the Sample Diluent/Negative Control. When using the transfer pipette, the sample is added directly into the Sample Diluent/Negative Control.

The diluted sample is mixed and dispensed drop-wise into the sample port of the Curian Campy test card. If present, the *Campylobacter* antigen binds to the monoclonal detector antibody conjugated to fluorescent particles, forming a complex. As the sample moves through the test strip, the anti-*Campylobacter* capture antibody, bound to the assay membrane at the test position of the strip, binds the complex and yields a test line. When antigen is not present, a complex is not formed, and a test line will not form. As the sample continues to move further up the test strip, the polyclonal capture antibody, bound to the assay membrane at the control position of the strip, binds the conjugated antibody and yields a control line. A line at the control position of the test strip should be present each time a sample or external control is tested. If the control line is not generated, adequate sample flow has not occurred, and the Curian Analyzer will consider the test invalid.

REAGENTS/MATERIALS PROVIDED

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

1. **Curian Campy Test Card:** A test strip enclosed in a plastic frame which is in a foil pouch with a desiccant. Supplied ready to use.
2. **Curian Campy Aioprep Sample Preparation Device/ Negative Control:** The Aioprep device is fitted with a metering insert and dropper tip. Supplied ready to use. A buffered protein solution containing blue dye and 0.094% sodium azide.
3. **Curian Campy Positive Control:** Inactivated *Campylobacter jejuni* antigen in a phosphate buffered solution containing 0.094% sodium azide. Supplied ready to use.
4. **Transfer Pipettes**

MATERIALS PROVIDED SEPARATELY

1. Curian Campy Stool Collection Brushes, Meridian Bioscience, Inc. Catalog 11516 (To order brushes, please contact Meridian's Technical Services Department at 1-800-343-3858 or your local distributor.)

MATERIALS NOT PROVIDED

1. Disposable gloves, powder free.
2. Wooden applicator sticks
3. Transport Media (Cary Blair or C&S)

EQUIPMENT NOT PROVIDED

1. Vortex mixer
2. Interval timer (Optional)
3. Curian Analyzer System, Meridian Bioscience, Inc. Catalog 610190

PRECAUTIONS

1. All reagents are for *in vitro* diagnostic use only.
2. Rx Only
3. Store the kit at the temperature indicated on labeling when not in use.
4. Do not interchange transfer pipettes, sample collection brushes, or Aiopreps between assays. Specimens must be sampled and prepared with the transfer pipettes or the Curian Campy sample collection brushes (provided as a separate accessory item) and used with the Curian Campy Aioprep device. These components are not interchangeable with other assays.
5. Handle and dispose of all human specimens as if they are biologically hazardous.
6. Inspect foil pouch before removing the test card. Do not use test cards that have holes in the foil pouch or where the pouch has not been completely sealed.
7. Do not use test cards where the desiccant indicator has changed from blue to pink.
8. Inspect Curian Campy Aioprep Sample Preparation Device/ Negative Control prior to use. Tap the Aioprep to ensure the liquid is in the main chamber of the Aioprep.
9. Do not interchange positive control, Aiopreps, or test cards between kit lots.
10. Do not mark over or near the barcode on the test card.
11. The Curian Campy test card must be incubated outside of a laminar flow hood; this can be done either inside the analyzer or on the lab bench. Sample preparation in the Aioprep can be performed inside a laminar flow hood.
12. Do not use transfer pipettes with formed and solid stool specimens.

HAZARD and PRECAUTIONARY STATEMENTS

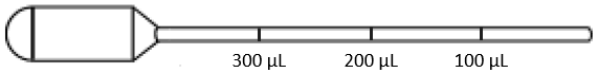
 **Aioprep Sample Preparation Device / Negative Control**	**Signal word** Danger **Hazard statements** H360 - May damage fertility or the unborn child **Precautionary Statements - EU (§28, 1272/2008)** P201 – Obtain special instructions before use P202 – Do not handle until all safety precautions have been read and understood P280 - Wear eye protection/ face protection P308 + P313 – IF exposed or concerned: Get medical device advice/attention P405 – Store locked up P501 – Dispose of contents/container to an approved waste disposal plant.

SHELF LIFE AND STORAGE

The expiration date is indicated on the kit box label. Store the kit at 2 - 8 C as indicated on the labeling. Return all kit components to the indicated storage temperature after use.

PROCEDURAL NOTES

The Curian Campy transfer pipette is diagrammed below:



OPERATION OF THE CURIAN ANALYZER SYSTEM

The Curian Analyzer is an easy to use, menu driven analyzer system. Instructions to complete testing are provided on the analyzer touchscreen and in the Curian Operator's Manual.

1. Power ON the Curian Analyzer by pressing the power button located on the left side of the analyzer. The Curian Analyzer will initialize and perform SELF TEST.
2. Instrument Checks, Quality Control testing, and Patient Specimen testing are completed by navigating from the Home screen to the test menus. User ID and Sample ID entries are required for Quality Control and specimen testing.
3. Complete use and operation of the Curian Analyzer System by following the instructions in the Curian Operator's Manual and on-screen prompts. **Refer to operator's manual for detailed instructions for the Curian Analyzer.**

SPECIMEN COLLECTION, HANDLING, AND STORAGE

This procedure is designed to be used with unpreserved stool or stool preserved in Cary-Blair or C&S transport media. DO NOT USE stool on swabs or mixed with fixatives. The patient specimen should be received in an airtight transport container and stored at 2 - 8 C until tested. The specimen should be tested as soon as possible, but preserved specimens may be held for up to 96 hours (four days) at room temperature (19 - 27 C), or unpreserved and preserved specimens (in Meridian Para-Pak C&S media) may be held for up to 7 days at 2 - 8 C prior to testing. If testing cannot be performed within this time frame, unpreserved specimens or specimens preserved in Meridian Para-Pak C&S media should be frozen immediately upon receipt and stored frozen for up to 59 days (or eight weeks) at ≤ -20 C until tested. Specimens may be frozen and thawed up to 5 times.

Specimen Condition	Storage Temperature	Maximum Storage Time
Unpreserved specimens	**2 – 8 C (refrigerated)**	**7 days**
	≤ -20 C (frozen)	**8 weeks**
Preserved specimens in Cary-Blair or C&S media	**19 – 27 C (room temperature)**	**96 hours**
	2 – 8 C (refrigerated)[1]	**7 days**
	≤ -20 C (frozen)[2]	**8 weeks**
Prepared specimens in Aioprep / Sample Diluent	**19 – 27 C (room temperature)**	**8 hours**
	2 – 8 C (refrigerated)	**24 hours**

[1] Refrigerated storage validated with Meridian Para-Pak C&S media only. If other C&S or Cary-Blair transport media are used, specimens should be stored according to the recommendations in the respective transport media package insert.

[2] Frozen storage validated with Meridian Para-Pak C&S media only. If other C&S or Cary-Blair transport media are used, specimens should be stored according to the recommendations in the respective transport media package insert.

SAMPLE PREPARATION AND TEST PROCEDURE

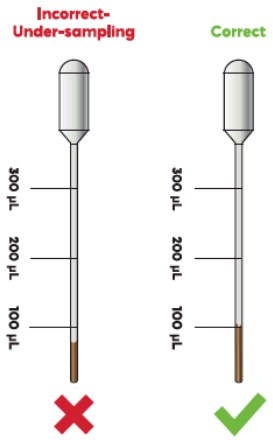
Note: Handle all waste and specimens as biohazardous.

Note: Before testing, mix stool specimen thoroughly by vortexing or inverting for liquid/semi-solid specimens, or with a wooden applicator stick for formed/solid specimens.

Bring all test components, reagents and specimens to 19 - 27 C before testing. Use one Curian Campy test card for each sample. When ready to perform testing, inspect the pouch for damage. If undamaged, remove test card from its foil pouch. Discard the pouch and desiccant. **Do not use if desiccant is pink. Do not cover or mark on the barcode.**

1. **Sample Preparation:**
   Prior to sampling, **thoroughly mix stool specimen** by vortexing for approximately 5 seconds or inverting (e.g., 3 - 4 times), or with a wooden applicator stick for formed/solid specimens. Remove the yellow top cap from the Aioprep device and set aside.
   Note: Prepared specimens in the Aioprep / Sample Diluent may be held at room temperature (19 - 27 C) for up to 8 hours or may be stored at refrigerated temperature (2 - 8 C) for up to 24 hours, prior to proceeding to step 2 for testing.

   Note: Ensure that the measurement markings on the transfer pipette are not obscured during sample aspiration.

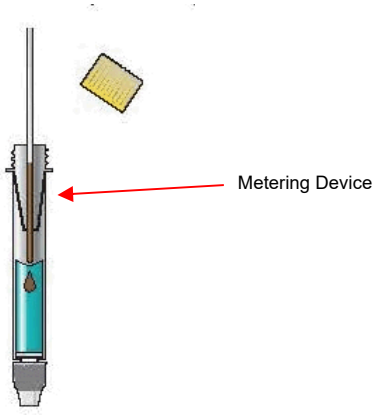


Pipettable, liquid/semi-solid specimens:

a. **Human stool specimens, preserved in Cary-Blair or C&S media:**
   i. Using the provided transfer pipette, transfer 300 µL of preserved stool (third mark from pipette tip) directly into the Sample Diluent by inserting the tip of the pipette through the metering insert while expelling specimen to ensure the stool is transferred. **NOTE: Transferring too little specimen, or failure to mix and thoroughly suspend the specimen in the Aioprep/ Sample Diluent, may result in a false-negative result. Refer to the examples below.** Recap the Aioprep device.
   ii. Proceed to step 2.

b. **Human stool specimens, unpreserved:**
   i. Using the provided transfer pipette, transfer 100 µL of unpreserved stool (first mark from pipette tip) directly into the Sample Diluent by inserting the tip of the pipette through the metering insert while expelling specimen to ensure the stool is transferred. **NOTE: Transferring too little specimen, or failure to mix and thoroughly suspend the specimen in the Aioprep/ Sample Diluent, may result in a false-negative result. Refer to the examples below.** Recap the Aioprep device.
   ii. Proceed to step 2.

User may encounter slight resistance when inserting tip of pipette through metering insert.



Non-pipettable, formed/solid specimens (either preserved in Cary-Blair or C&S media or unpreserved):

a. Using the Curian Campy sample collection brush, insert the brush into the stool specimen to coat the bristles of the brush. It is important that only the brush is covered with stool. Do not get stool on the shaft or handle of the brush; wipe any excess stool on the inside of the specimen container. Refer to the examples below.

Correct

Incorrect- Under-sampling

Incorrect- Over-sampling

b. Insert the Curian Campy sample collection brush into the Aioprep device, pushing the brush through the metering insert. Snap off the sample collection brush at the marked 'Break' point and discard the handle. Recap the Aioprep device, leaving the Curian Campy sample collection brush in the device.
c. Proceed to step 2.

2. **Do not invert or shake Aioprep.** Thoroughly mix the sample in the Aioprep sample diluent by vortexing for approximately 5 seconds. **Sample must be thoroughly suspended in the Aioprep sample diluent.** Remove the white tip cap from the bottom of the Aioprep and discard. While holding the Aioprep vertically, squeeze in the center of the barrel to add 3 drops into the 'SAMPLE' port of the test card. Discard the Aioprep device immediately.

3. Start the Test Read by navigating to the 'TEST' Menu, select 'ANALYZE NOW' or 'INCUBATE AND ANALYZE', and then follow the on-screen instructions.
For Analyze Now:
   a. Incubate the test card at 19 - 27 C on the benchtop for 20 minutes.
   b. Enter Sample ID and press Ok.
   c. Within 2 minutes of the end of incubation, insert the test card into the drawer of the Curian Analyzer and close the drawer. Analysis of the reaction will be initiated by the Curian Analyzer.
   d. The Curian Analyzer will analyze the test card and automatically report the test result.
For Incubate and Analyze:
   a. Enter Sample ID and press Ok.
   b. Immediately insert the test card into the drawer of the Curian Analyzer and close the drawer. The analyzer will time the incubation for 20 minutes.
   c. After the end of the incubation, the Curian Analyzer will analyze the test card and automatically report the test result.

ASSAY EXTERNAL QUALITY CONTROL (QC) TEST PROCEDURE

Note: Good Laboratory Practice (GLP) guidelines[9] recommend the use of control material. Users should follow the appropriate federal, state, and local guidelines concerning the running of external quality controls.

Bring all test components and reagents to 19 - 27 C before testing. Use one Curian Campy test card for each external QC test (Negative and Positive Controls). When ready to perform testing, remove the test card from its foil pouch. Discard the pouch and desiccant. **Do not use if desiccant is pink. Do not mark over the barcode on the test card.**

Start the Test Read by navigating to the 'TEST' Menu, selecting the 'QC TEST', and then selecting 'ANALYZE NOW' or 'INCUBATE AND ANALYZE'. Follow the on-screen instructions.

Negative Control

1. Remove the white tip cap from the bottom of the Aioprep and discard. While holding the Aioprep vertically, squeeze in the center of the barrel to add 3 drops of Negative Control reagent (Sample Diluent) into the 'SAMPLE' port of the test card. Discard the Aioprep device immediately.
2. **For Analyze Now**:
   a. Incubate the test card at 19 - 27 C on the benchtop for 20 minutes.
   b. Enter Sample ID and press Ok.
   c. Within 2 minutes of the end of incubation, insert the test card into the drawer of the Curian Analyzer and close the drawer. Analysis of the reaction will be initiated by the Curian Analyzer.
   d. Select 'Curian Campy QC Negative'.
   e. The Curian Analyzer will analyze the test card and automatically report the test result.

   For Incubate and Analyze:
   a. Enter Sample ID and press Ok.
   b. Insert test card immediately into the Curian Analyzer.
   c. Select 'Curian Campy QC Negative'.
   d. The Curian Analyzer will time the incubation for 20 minutes, then read the test card and automatically report the test result.

Positive Control

1. Invert the positive control bottle to mix. Remove the Positive Control tip cap. Holding vertically, squeeze 3 drops of Positive Control into the 'SAMPLE' port of the test card.
2. **For Analyze Now:**
   a. Incubate the test card at 19 - 27 C on the benchtop for 20 minutes.
   b. Enter Sample ID and press Ok.
   c. Within 2 minutes of the end of incubation, insert the test card into the drawer of the Curian Analyzer and close the drawer. Analysis of the reaction will be initiated by the Curian Analyzer.
   d. Select 'Curian Campy QC Positive'.
   e. The Curian Analyzer will analyze the test card and automatically report the test result.

   For Incubate and Analyze:
   a. Enter Sample ID and press Ok.
   b. Insert the test card immediately into the drawer of the Curian Analyzer and close the drawer.
   c. Select 'Curian Campy QC Positive'.
   d. The Curian Analyzer will time the incubation for 20 minutes, then read the test card and automatically report the test result.

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. Do NOT perform patient testing if either of the QC test results are incorrect.

INSTRUMENT CHECK (IC) TEST PROCEDURE

Note: The analyzer's External Control (IC Test) has a default schedule of 30 days. The IC Test will be required to be performed prior to running a QC test or patient test if this has not been performed within this timeframe. The user will be locked-out of running a QC test or patient test if this test is overdue.

1. Power ON the Curian Analyzer by pressing the power button located on the side of the Curian Analyzer. The analyzer will initialize and perform SELF TEST (automatic) checks.
2. From the HOME screen, select 'TEST', and then select 'INSTRUMENT CHECK'.
3. Insert the Fluorescent IC Card into the drawer. A 'PASS' or 'FAIL' result will be displayed once the analysis is complete.

If the expected result is not observed, repeat the IC Test as the first step in determining the root cause of the failure. Refer to the Curian Analyzer Operator's Manual for detailed instructions for the analyzer.

INTERPRETATION OF RESULTS

Result interpretation is completed automatically by the Curian Analyzer system. The result will be shown on screen. Results can be retrieved from analyzer storage, printed and/or exported.

Patient Test Results:

One of the following result interpretations will be generated by the Curian Analyzer for patient specimens.

1. **Positive:** *Campylobacter* antigen is present in sample.
2. **Negative:** *Campylobacter* antigen is not present in sample**.**
3. **Control Invalid:** Result indicates inadequate flow of sample. Repeat the test from the original stool specimen. If repeat testing yields the same results, please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor for additional assistance.

External Quality Controls:

One of the following result interpretations will be generated by the Curian Analyzer for External Controls.

1. **Pass:** This indicates the test card and reagents are performing as intended.
2. **Fail**: This result indicates the test card and/or reagents are not performing as intended or a user error has occurred. Test should be repeated to assist in trouble-shooting the error. If repeat testing results in a failed output, please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor for additional assistance. **Do NOT perform patient testing if either of the QC test results are incorrect**.
3. **Control Invalid**: This result indicates inadequate sample flow. Repeat the test. If repeat testing yields the same results, please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor for additional assistance.

Instrument Check:

One of the following result interpretations will be generated by the Curian Analyzer for Instrument Check.

1. **Pass:** This indicates that the essential and critical components of the Curian Analyzer are working correctly.
2. **Fail**: This indicates that there may be an issue with the Curian Analyzer. Refer to the Curian Operator's Manual if an error message has occurred. Repeat testing to assist in trouble-shooting the error. If repeat testing results in a failed output, please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor for additional assistance.

QUALITY CONTROL
There are four controls for the Curian Campy test system. There is an external and internal control for both the analyzer and assay.

1. Analyzer Internal Control: Self Test (automatic)
2. Analyzer External Control: Instrument Check (IC) Test
3. Assay External Controls: Positive and Negative Controls (QC test)
4. Assay Internal Control: Control Line

EXPECTED VALUES
The prevalence of *Campylobacter* infection observed during the 2020 study period for prospectively collected specimens was 1.2% (18/1474). The prevalence of *Campylobacter spp.* by sex assigned at birth for the tested population is provided below. The age demographic for the tested population ranged from less than 1 year old to 99 years old, with an average age of 48 years old.

Sex Assigned at Birth	Samples Positive by Curian Campy and Reference Method	Prevalence in Tested Population
Males (n=578)	10	1.7%
Females (n=896)	8	0.9%
Overall (n=1474)	18	1.2%

LIMITATIONS OF THE PROCEDURE
1. The Curian Campy assay must only be used with the Curian Analyzer.
2. Curian Campy is a qualitative, *in vitro* diagnostic test. The Curian Analyzer will only provide qualitative results. This test is not intended to provide quantitative results.
3. Curian Campy detects both viable and nonviable *Campylobacte*r bacteria and may yield a positive result in the absence of living organisms.
4. A negative test result does not definitively rule-out the presence of *Campylobacter* species in suspected patients. Levels of organism may be present in feces beneath the limit of detection for the Curian Campy assay, and therefore, if *Campylobacter* is suspected, alternative testing should be conducted.
5. A negative result may occur if the sample was collected, transported, or stored improperly.
6. Failure to follow the test procedure may adversely affect test performance and/or invalidate the test result.
7. Test results must be evaluated in conjunction with other clinical data available to the physician.
8. Negative test results do not rule out other possible infections.
9. Positive test results do not rule out co-infections with other pathogens.
10. Curian Campy is designed to be used with unpreserved stool samples or stools preserved in Cary-Blair or C&S media. DO NOT USE stool on swabs or in other transport media (e.g., formalin, polyvinyl alcohol). No data exists on the effects of colonic washes, barium enemas, laxatives, or bowel preparations on the performance of Curian Campy. All of these procedures can result in extensive dilution or the presence of additives that may affect test performance.
11. Transferring too little specimen, or failure to mix and completely suspend the specimen in the Sample Diluent, may result in a false negative test result.
12. The performance of this test has not been evaluated for use in patients without signs and symptoms of gastroenteritis.
13. *Campylobacter helveticus* (strain ATCC 51209) at levels greater than 3.75 x 10^6 CFU/mL in unpreserved stool or greater than 7.50 x 10^6 CFU/mL in preserved stool may cross react or interfere with the performance of the test.
14. Non-pipettable, formed/solid specimens should only be processed with the Curian Campy Sample Collection Brush (Meridian Bioscience, Inc. Catalog 11516).

SPECIFIC PERFORMANCE CHARACTERISTICS
CLINICAL PERFORMANCE

Prospective Study
The Curian Campy assay was evaluated from July 2020 to December 2020 at five clinical study sites representing geographically distinct regions throughout the United States. There were 1,474 specimens from patients with signs and symptoms of gastroenteritis for whom a diagnostic Campylobacter test had been ordered by a practicing physician, prospectively collected and enrolled into the study. All specimens were tested at the study sites with the Curian Campy assay and either had current standard of care *Campylobacter* culture and speciation performed and results available (reference method) or culture and speciation was performed as part of the study. The vast majority of specimens were pipettable and were processed with the Curian Campy pipette included in the kit. A small subset of specimens were non-pipettable and were processed with the Curian Campy Collection Brush (sold separately by Meridian). Clinical performance (sensitivity and specificity) for prospective specimens against the reference method (culture and speciation) are presented in the following table. There were no observable differences in performance of the Curian Campy assay with respect to study site, storage condition, kit lot, or patient gender or age. Prospective specimens with discordant results between the Curian Campy assay and the reference method were further evaluated using standard of care (SoC) results obtained with an FDA-cleared commercial nucleic acid amplification test (NAAT); results of discordant testing are footnoted below.

Curian Campy Overall Performance for Prospective Specimens versus Culture and Speciation

		Reference Method: Culture and Speciation					
		Positive	Negative	Total	Parameter	Estimate	95% CI
Curian Campy Assay	Positive	18	28**	46	Sensitivity	85.7%	[65.4% - 95.0%]
	Negative	3*	1425	1428	Specificity	98.1%	[97.2% - 98.7%]
	Total	21	1453	1474			

* The Standard of Care (SoC) testing of two of the three false negative specimens by a high sensitivity, FDA-cleared nucleic acid amplification test (NAAT) assay showed that one of the specimens produced a negative Campylobacter result, whereas one of the specimens produced a positive Campylobacter result; the third specimen was not subjected to NAAT testing as part of the SoC.

** Of the 28 false positive specimens, 10 were subjected to SoC testing by a high sensitivity, FDA-cleared NAAT assay. Two of these 10 specimens produced a positive Campylobacter result, whereas eight produced a negative Campylobacter result. Eighteen specimens were not subjected to NAAT testing as part of the SoC.

Archived Study
To further estimate sensitivity and specificity of the Curian Campy assay, 290 archived specimens with culture and speciation results were retrospectively tested for *Campylobacter* antigen using the Curian Campy assay at all five study sites. The clinical performance (sensitivity and specificity) for archived specimens against the reference method (culture and speciation) are presented in the table below.

Curian Campy Overall Performance for Archived Specimens versus Culture and Speciation

		Reference Method: Culture and Speciation					
		Positive	**Negative**	**Total**	**Parameter**	**Estimate**	**95% CI**
Curian Campy Assay	**Positive**	28	5	33	Sensitivity	96.6%	[82.8% - 99.4%]
	Negative	1	256	257	Specificity	98.1%	[95.6% - 99.2%]
	Total	29	261	290			

Contrived Study

Additional testing at each site was conducted with contrived samples at 2xLoD and 8xLoD for *C. coli*, *C. upsaliensis*, and *C. lari* and 3 reagent lots. All 210 samples tested as expected with n=150 positive *Campylobacter* spp. samples and n=60 negative samples yielding 100% correlation with the anticipated results. Both sample matrices were represented with n=105 unpreserved stool samples and n=105 stool samples preserved in C&S media. The overall performance of the Curian Campy assay compared to the anticipated results is presented in the table below.

Curian Campy Overall Performance for Contrived Samples versus Contrived Anticipated Results

		Contrived: Anticipated Result					
		Positive	**Negative**	**Total**	**Parameter**	**Estimate**	**95% CI**
Curian Campy Assay	**Positive**	150	0	150	PPA	100.0%	[97.5% - 100.0%]
	Negative	0	60	60	NPA	100.0%	[94.0% - 100.0%]
	Total	150	60	210			

ANALYTICAL SENSITIVITY

Analytical sensitivity studies were performed to determine the analytical limit of detection (LoD) of quantified *Campylobacter* whole cell stocks (*C. jejuni*, *C. coli*, *C. upsaliensis*, and *C. lari*) in human stool matrix for the Curian Campy assay. The LoD is defined as the lowest concentration of the target analyte that produces positive results ≥ 95% of the time.

The LoD values determined for the Curian Campy assay for each intended species in unpreserved and preserved (C&S, Cary Blair) stool matrix are listed below.

C. jejuni		***C. coli***		***C. upsaliensis***		***C. lari***	
CFU/mL	CFU/test	CFU/mL	CFU/test	CFU/mL	CFU/test	CFU/mL	CFU/test
Unpreserved Stool Matrix							
$4.00x10^5$	1818	$3.00x10^6$	13636	$1.62x10^6$	7386	$5.00x10^6$	22727
Preserved (C&S) Stool Matrix							
$7.25x10^5$	2266	$1.57x10^7$	49063	$1.18x10^6$	3681	$1.16x10^7$	36250
Preserved (Cary Blair) Stool Matrix							
$7.25x10^5$	2266	$1.57x10^7$	49063	$2.36x10^6$	7375	$1.16x10^7$	36250

ASSAY REACTIVITY/ INCLUSIVITY

Several strains of *C. jejuni*, *C. coli*, *C. upsaliensis*, and *C. lari* were used to evaluate the specificity of the Curian Campy assay using both unpreserved and preserved stool (in C&S).

All strains listed below generated positive results when tested.

C. jejuni	***C. coli***	***C. upsaliensis***	***C. lari***
Zeptometrix Z086	ATCC 43482	ATCC BAA-1059	ATCC BAA-1060
ATCC 33292	ATCC 43476	ATCC 49815	ATCC 35222*
ATCC 49350	ATCC 43478	2017/0506H	ATCC 35223*
ATCC 43442	ATCC 43485	2016/1950*	2013/0823H*
ATCC 33560	ATCC BAA-1061	ATCC 43954	2015/0814*
		ATCC 43953*	2015/2983
		2016/2697*	2016/0235*
		2018/0319H*	2015/0519
		2016/1931	2015/1582
		ATCC 700558*	2015/2189

*The following *C. upsaliensis* and *C. lari* strains exhibited elevated LoDs in comparison to the reference strains (ATCC 49816 and ATCC 43675) that were used in the LoD determination:

Species	**Strain**	**Unpreserved Stool**	**Stool Preserved in C&S**
C. upsaliensis	2016/1950	4x	8x
	ATCC 43953	2x	8x
	2016/2697	56x	78x
	2018/0319H	40x	55x
	ATCC 700558	24x	33x
C. lari	2013/0823H	8x	4x
	2015/0814	8x	4x
	2016/0235	8x	10x
	ATCC 35222	8x	10x
	ATCC 35223	24x	10x

REPRODUCIBILITY

Ten manufactured reference panels per sample type (unpreserved stool and stool preserved in C&S transport media) were supplied to three laboratories for this reproducibility study. The panels each contained sixteen blinded samples, including five high-negative samples (just below C_5) , five low-positive samples (~C_{95}), five moderate-positive samples (3x higher than the C_{95}), and one true negative sample. Panel members were manufactured by spiking *Campylobacter jejuni* organism into pooled negative stool matrix. Positive and negative external controls were run daily.

Different operators, per site, tested each panel type per day for five days, totaling 320 samples (160 unpreserved stools and 160 stools preserved in C&S transport media). Each operator ran the external controls each day of testing. Three lots of Curian Campy kits and four Curian Analyzers were used in this study. The results of the study are provided in the table below.

		Site 1 - Percent Agreement		Site 2 - Percent Agreement		Site 3 - Percent Agreement		Total Percent Agreement	
Sample Type	**Sample Category**	**Rate**	**%**	**Rate**	**%**	**Rate**	**%**	**Rate**	**%**
Controls	**Negative Control**	10/10	100.0	11/11	100.0	20/20	100.0	41/41	100.0
	Positive Control	10/10	100.0	11/11	100.0	20/20	100.0	41/41	100.0
Preserved in C&S media	**True Negative**	10/10	100.0	10/10	100.0	10/10	100.0	30/30	100.0
	High Negative	45/50	90.0	48/49[1]	98.0	49/50	98.0	142/149	95.3
	Low Positive	50/50	100.0	50/50	100.0	50/50	100.0	150/150	100.0
	Moderate Positive	50/50	100.0	50/50	100.0	50/50	100.0	150/150	100.0
Unpreserved Stool	**True Negative**	10/10	100.0	10/10	100.0	10/10	100.0	30/30	100.0
	High Negative	48/50	96.0	50/50	100.0	50/50	100.0	148/150	98.7
	Low Positive	50/50	100.0	41/50	82.0	33/50	66.0	124/150	82.7
	Moderate Positive	50/50	100.0	50/50	100.0	50/50	100.0	150/150	100.0

[1] A single replicate was not performed because of instrument error and there was insufficient volume for a repeat test.
[2] For the unpreserved low positive panel member, Percent Agreement (PA) was lower than expected; under-sampling with the Curian Campy transfer pipette was the cause.

CROSS-REACTIVITY/ MICROBIAL INTERFERENCE

The Curian Campy assay was evaluated for cross-reactivity and microbial interference with the organisms listed below. Unless otherwise indicated, each organism was tested at minimum concentrations of 1.0 x 10^7 CFU/mL for bacteria/fungi or 1.0 x 10^5 $TCID_{50}$/mL for viruses. The assay's reactivity with Norovirus was evaluated using clinical Norovirus-positive stool specimens. None of the organisms showed cross-reactivity or microbial interference in the Curian Campy assay, except for *Campylobacter helveticus*, which was found to be positive at concentrations greater than 3.75 x 10^6 CFU/mL in unpreserved stool and 7.50 x 10^6 CFU/mL in preserved stool.

Acinetobacter baumannii
Aeromonas hydrophila
Bacillus cereus
Bacillus subtilis
Bacteroides fragilis
Campylobacter concisus
Campylobacter fetus
Campylobacter helveticus
Campylobacter hyointestinalis
Candida albicans
Citrobacter freundii
Clostridium bifermentans
Clostridium difficile
Clostridium perfringens
Edwardsiella tarda
Enterobacter cloacae
Enterococcus faecalis
Escherichia coli
Escherichia coli EIEC
Escherichia coli EPEC
Escherichia coli ETEC
Escherichia coli O157:H7 (non-toxigenic)
Escherichia coli O157:H7 (toxigenic)
Escherichia fergusonii
Escherichia hermanii
Helicobacter pylori
Klebsiella pneumoniae
Lactobacillus acidophilus
Lactococcus lactis
Listeria monocytogenes
Peptostreptococcus anaerobius
Plesiomonas shigelloides
Porphyromonas asaccharolytica
Prevotella melaninogenica
Proteus vulgaris
Pseudomonas aeruginosa
Pseudomonas fluorescens
Salmonella enterica susb. enterica serovar Hilversum
Salmonella enterica susb. enterica serovar Typhimurium
Salmonella enterica susb. enterica serovar Minnesota
Serratia marcescens
Shigella boydii
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Staphylococcus aureus
Staphylococcus aureus (Cowan's)
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus dysgalactiae subsp. equisimilis
Vibrio parahaemolyticus
Yersinia enterocolitica

Adenovirus Type 1, 2, 3, 5, 40, 41
Coxsackievirus B2, B3, B4, B5
Echovirus 9, 11, 18
Enterovirus 68, 69, 70, 71
Human Coronavirus
Human Rotavirus
Norovirus
Parechovirus 1 (formerly Echovirus 22)

TESTS FOR INTERFERING SUBSTANCES

The chemical and biological substances listed below were evaluated at the indicated concentrations for interference in the Curian Campy assay. None of the substances showed interference with the Curian Campy assay performance.

Barium Sulfate (5% w/v)
Benzalkonium chloride (1% w/v)
Ciprofloxacin (0.25% w/v)
Ethanol (1% w/v)
Hog gastric mucin (3.5% w/v)
Human blood (40% v/v)
Human hemoglobin (10.0% w/v)
Human urine (5% v/v)
Hydrocortisone (1% w/v)
Imodium® A-D (5% v/v)
Kaopectate® (5% v/v)
Leukocytes (0.05% v/v)
Mesalazine (10% w/v)
Metronidazole (0.25% w/v)
Mineral Oil (10% w/v)
Mylanta® (4.2 mg/mL)
Naproxen sodium (5% w/v)
Nonoxynol-9 (1% w/v)
Nystatin (1% w/v)
Palmitic Acid/Fecal Fat (40% w/v)
Pepto-Bismol® (5% v/v)
Phenylephrine (1% w/v)
Polyethylene glycol 3350 (10% w/v)
Prilosec OTC® (5 µg/mL)
Sennosides (1% w/v)
Simethicone (10% w/v)
Stearic Acid/Fecal Fat (40% w/v)
Tagamet® (5 µg/mL)
TUMS® (50 µg/mL)
Vancomycin (0.25% w/v)

BRUSH BRIDGING STUDY

The Curian Campy assay is for use with unpreserved human stool specimens and human stool specimens preserved in Cary-Blair and C&S transport media. Most specimens are easily sampled using the transfer pipette provided in the kit; however, some unpreserved stool specimens are non-pipettable and require use of a specific brush (i.e., Curian Campy Stool Collection Brush, sold separately by Meridian) to adequately collect the sample for analysis in the Curian Campy assay. Fifty-three non-pipettable stool specimens (5 *Campylobacter* positives and 48 negatives) were processed with the brush during the prospective and archived clinical studies, combined.

To further support use of the brush with the Curian Campy assay, an analytical bridging study was performed that evaluated a panel consisting of contrived positive and negative samples collected/processed with the brush. Contrived positive samples were generated by spiking *C. jejuni* (strain ATCC #BAA-1234) into negative, non-pipettable stool samples. Ten samples were prepared at 3x LoD and 25 samples were prepared at 5x LoD. Twenty-five negative samples were also tested. Three non-expert operators tested each sample at one internal site. All positive samples gave the expected positive results, and all negative samples were negative. These results indicate that non-pipettable stool specimens can be collected with the brush prior to testing with the Curian Campy assay.