**Curian Campy** Method Validation Protocol

#### Overview

* 1. **Introduction:** 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.
  2. **Objective:**

The purpose is to validate the Curian Campy assay for *Campylobacter* testing. Method validation will be performed to meet the requirements of establishing accuracy, analytical sensitivity, precision, and reportable range.

* 1. **Description:**

Method validation of test accuracy will consist of testing a minimum of 25 specimens. Ten positive and ten negative specimens will be provided by the manufacturer and at least five patient specimens will be compared to the current method. To verify test precision, a known positive and known negative specimen will be tested for three days by a minimum of two different operators.

Data on analytical sensitivity and interfering substances are provided in the manufacturer’s Package Insert and are accepted and adopted.

* 1. **Accuracy:**

A panel of ten known positive and ten known negative specimens provided by the manufacturer will be tested. Results will be recorded in Table 1. A minimum of five patient specimens will be tested and results compared to the current testing method. Results will be recorded in Table 2.

* 1. **Reproducibility or Precision:**

Intra and Inter assay precision will be performed over three days. Intra assay precision will be verified by testing a known positive and a known negative specimen in duplicate in the same run. Inter assay precision will be verified by repeating the known positive and known negative for two additional days by a minimum of two different operators. Results will be recorded in Table 3.

1. Day one (training day): Test specimens CAM-1 and CAM-2 in duplicate in the same run.
   1. Store specimens in 2-8 °C
2. Day two: An operator that did not test on day one, repeat testing of CAM-1 and CAM-2.
   1. Store specimens in 2-8 °C
3. Day three: Any trained operator, repeat specimens CAM-1 and CAM-2.
   1. **Reportable Range:**

The Curian Campy assay is a qualitative test. The reportable range is positive for *Campylobacter* or negative for *Campylobacter*. No quantitative values are reported.

* 1. **Analytical Sensitivity:**

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** | | | | | | | |
| 4.00x105 | 1818 | 3.00x106 | 13636 | 1.62x106 | 7386 | 5.00x106 | 22727 |
| **Preserved (C&S) Stool Matrix** | | | | | | | |
| 7.25x105 | 2266 | 1.57x107 | 49063 | 1.18x106 | 3681 | 1.16x107 | 36250 |
| **Preserved (Cary Blair) Stool Matrix** | | | | | | | |
| 7.25x105 | 2266 | 1.57x107 | 49063 | 2.36x106 | 7375 | 1.16x107 | 36250 |

* 1. **CROSSREACTIVITY STUDIES** as stated in the Curian CAMPY Package Insert:

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 107 CFU/mL for bacteria/fungi or 1.0 x 105 TCID50/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 106 CFU/mL in unpreserved stool and 7.50 x 106 CFU/mL in preserved stool.

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

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

* 1. **TESTS FOR INTERFERING SUBSTANCES** as stated in the Curian CAMPY Package Insert.

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

**Validation Result Sheets**

**Table 1 - Accuracy Testing**

Specimen Panel Lot# \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Kit Lot#/Exp. date\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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| --- | --- | --- | --- | --- |
| Specimen ID | Expected Result | Valid (Y/N) | Actual Result | Performed By |
| Positive Control | Positive |  |  |  |
| Negative Control | Negative |  |  |  |
| CAM-1 | Negative |  |  |  |
| CAM-2 | Positive |  |  |  |
| CAM-3 | Negative |  |  |  |
| CAM-4 | Positive |  |  |  |
| CAM-5 | Negative |  |  |  |
| CAM-6 | Positive |  |  |  |
| CAM-7 | Negative |  |  |  |
| CAM-8 | Positive |  |  |  |
| CAM-9 | Negative |  |  |  |
| CAM-10 | Positive |  |  |  |
| CAM-11 | Negative |  |  |  |
| CAM-12 | Positive |  |  |  |
| CAM-13 | Negative |  |  |  |
| CAM-14 | Positive |  |  |  |
| CAM-15 | Negative |  |  |  |
| CAM-16 | Positive |  |  |  |
| CAM-17 | Negative |  |  |  |
| CAM-18 | Positive |  |  |  |
| CAM-19 | Negative |  |  |  |
| CAM-20 | Positive |  |  |  |

**Table 2 – Accuracy testing**

**Patient Specimen Results Sheet**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Patient ID | Current Test Method Result | Curian CAMPY Result | Internal Control Valid (Y/N) | Performed by/Date | Discrepant Y/N |
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**Table 3 – Precision Validation**

**Specimen Panel Lot# \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_** **Kit Lot#/Exp. Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Date | Specimen ID | Expected Result | Valid (Y/N) | Actual Result | Performed by |
| Day 1 |  | **CAM-1** | Negative |  |  |  |
| **CAM-2** | Positive |  |  |  |
| **CAM-1** | Negative |  |  |  |
| **CAM-2** | Positive |  |  |  |
| Day 2 |  | **CAM-1** | Negative |  |  |  |
| **CAM-2** | Positive |  |  |  |
| Day 3 |  | **CAM-1** | Negative |  |  |  |
| **CAM-2** | Positive |  |  |  |

This validation study has been reviewed and the performance of the method is considered acceptable for patient testing.

*Reviewed By: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

*(Print name)*

*Approved by: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

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