* 1. **Introduction:**

Curian Shiga Toxin, for use with the Curian Analyzer, is a rapid, qualitative, fluorescent immunoassay for the detection of *Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2)* in human stool. The stool antigen test is intended to aid in the diagnosis Escherichia coli (STEC) infections and to demonstrate loss of *Shiga Toxins* following treatment.

* 1. **Objective:**

The purpose is to validate Curian Shiga Toxin® for *Escherichia coli (STEC)* testing. Method Validation will be performed to establish accuracy, precision, analytical sensitivity, reportable range, and interfering substances.

* 1. **Description:**

Method validation of test accuracy will include testing a minimum of twenty-five specimens. 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 compared to the results provided by the manufacturer. 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:**

To show results are reproducible, intra and inter assay precision testing will be performed. 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 same known positive and known negative for two additional days by a minimum of two different operators. Results will be recorded in Table 3.

1. Initial Test: Test specimens GN-1 and GN -20 in duplicate in the same run.
   1. Store specimens in 2-8 °C.
2. Second testing event: An operator that did not perform the initial intra assay testing will repeat specimens GN-1 and GN -20.
   1. Store specimens in 2-8 °C.
3. Third testing event: On a day different from the first two precision testing events, a trained operator will repeat testing of specimens GN-1 and GN -20.
   1. **Reportable Range:**

Curian Shiga Toxin is a qualitative test. The reportable range is positive and negative. No quantitative values will be reported.

* 1. **Analytical Sensitivity:**

The LoD is defined as the lowest concentration of measurand which produced positive results ≥ 95% of the time. The analytical sensitivity as stated in the CurianShiga Toxin package insert is 0.185 ng/mL for Stx1 and 0.125 ng/mL for Stx2.

* 1. **TESTS FOR INTERFERING SUBSTANCES** as stated in the Curian *Shiga Toxin*package insert.

The following substances were found to have no effect on results when present in stool at the concentrations indicated.

Barium Sulfate (5% w/v),Ciprofloxacin (0.25% 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),Imodium® A-D (5% v/v),Kaopectate® (5% v/v),Leukocytes (0.05% v/v),Mylanta® (8.400 mg/mL),Palmitic Acid/Fecal Fat (40% w/v),Pepto-Bismol® (5% v/v),Prilosec OTC® (5 μg/mL),Stearic Acid/Fecal Fat (40% w/v),Tagamet® (5 μg/mL),TUMS® (50 μg/mL),Naproxen sodium (0.5% w/v),Metronidazole (0.25% w/v),Vancomycin (0.25% w/v)

* 1. **CROSSREACTIVITY STUDIES** The Curian Shiga Toxin 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. None of the organisms showed cross-reactivity or microbial interference in the Curian Shiga Toxin assay.*.* *Aeromonas hydrophila,Gardnerella vaginalis,Bacillus subtilis,Helicobacter pylori,Bacteroides fragilis,Klebsiella pneumoniae,Campylobacter coli,Lactobacillus acidophilus,Campylobacter concisus,Proteus vulgaris,Campylobacter fetus,Providencia stuartii,Campylobacter hyointestinalis,Pseudomonas aeruginosa,Campylobacter jejuni,Pseudomonas fluorescens,Candida albicans,Salmonella enterica susb. enterica serovar Hilversum,Citrobacter freundii,Salmonella enterica susb. enterica serovar Typhimurium,Clostridium difficile,Salmonella minnesota,Clostridium perfringens,Serratia liquefaciens,Enterobacter cloacae,Serratia marcescens,Enterococcus faecalis,Shigella boydii,Escherichia coli (non-toxigenic),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 fergusonii,Streptococcus equisimilis subsp. dysgalactiae,Escherichia hermanii,Yersinia enterocolitica,Human Adenovirus 2,Human Coxsackievirus B1,Human Adenovirus 14,Human Enterovirus 69,Human Adenovirus 40,Human Rotavirus,Human Adenovirus 41,Feline calicivirus,Human Coxsackievirus A9*

*Shigella species shown to be reactive with the Curian Shiga Toxin assay:*

*S. dysenteriae (strain ATCC 9361) was found to be Shiga toxin 1 (Stx1) positive at concentrations greater than 1.25 x 106 CFU/mL in the Curian Shiga Toxin assay.*

**Table 1 - Accuracy Testing Results**

Kit Lot Number: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Expiration Date:­­­­­­­\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Specimen Panel Lot#: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Table 1 – Accuracy Testing**

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

**Table 2 - Patient Specimen Results Sheet**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Patient ID | Current Test Method Result | Curian Shiga Toxin Result | Discrepant (Y/N) | Tech | Date |
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**Table 3 - Precision Validation**

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| --- | --- | --- | --- | --- | --- | --- |
|  | Date | Specimen ID | Expected Result | Internal Control Valid (Y/N) | Actual Result | Tech |
| Day 1 |  | **GN-1** | Negative |  |  |  |
| **GN-20** | Toxin 1 & 2 Positive |  |  |  |
| **GN-1** | Negative |  |  |  |
| **GN-20** | Toxin 1 & 2 Positive |  |  |  |
| Day 2 |  | **GN-1** | Negative |  |  |  |
| **GN-20** | Toxin 1 & 2 Positive |  |  |  |
| Day 3 |  | **GN-1** | Negative |  |  |  |
| **GN-20** | Toxin 1 & 2 Positive |  |  |  |

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

Reviewed By/Date: *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

*(Print name)*

Approved by/Date: *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

*(Signature)*

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*(Title*)