* 1. **Introduction:**

Curian HpSA, for use with the Curian Analyzer, is a rapid, qualitative, fluorescent immunoassay for the detection of *Helicobacter pylori* antigens in human stool. The stool antigen test is intended to aid in the diagnosis of *H. pylori* infection and to demonstrate loss of *H. pylori* stool antigen following treatment.

* 1. **Objective:**

The purpose is to validate Curian HpSA® for *H. pylori* 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 HPSA-1 and HPSA-2 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 HPSA-1 and HPSA-2.
   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 HPSA-1 and HPSA-2.
   1. **Reportable Range:**

Curian HpSA 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 CurianHpSA package insert is 2.0 ng/mL.

* 1. **TESTS FOR INTERFERING SUBSTANCES** as stated in the Curian *HpSA*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 (50 mg/ml)) Benzalkonium chloride (1% v/v) Ciprofloxacin (0.25% w/v (2.5 mg/ml)) Ethanol (1% v/v) Hog gastric mucin (3.5% w/v (125 mg/ml)) Human urine (5% v/v) Hydrocortisone (1% w/v (10 mg/ml)) Imodium (Loperamide HCl 1 mg/7.5 ml) (5% v/v) Kaopectate (Bismuth subsalicylate 262 mg/15 ml) (5% v/v) Leukocytes (0.05% v/v) Mesalazine (5-Aminosalicylic acid) (10% w/v (100 mg/ml)) Metronidazole (0.25% w/v (2.5 mg/ml)) MiraLAX (Polyethylene Glycol 3350, 17 g/dose) (7% w/v (70 mg/ml) Mineral Oil (10% v/v) Mylanta (per 10 ml: (Aluminum hydroxide 800 mg, Magnesium hydroxide 800 mg, Simethicone 80 mg) (4.2 mg/ml (2.5% v/v)) Naproxen Sodium (5% w/v (50 mg/ml)) Nonoxynol-9 (1% v/v) Nystatin (1% w/v (10 mg/ml)) Palmitic acid (fecal fat) (20% w/v (200 mg/ml)) Pepto-Bismol (Bismuth subsalicylate 525 mg/30 ml) (5% v/v) Phenylephrine (1% w/v (10 mg/ml)) Prilosec OTC (Omeprazole 20 mg/tablet) (5 mg/ml) Sennosides (1% w/v (10 mg/ml)) Simethicone (10 % v/v) Stearic acid (fecal fat) (20% w/v (200 mg/ml)) Tagamet HB 200 (Cimetidine 200 mg/tablet) (5 mg/ml) TUMS (5 mg/ml) Vancomycin (0.25% w/v (2.5 mg/ml))

* 1. **CROSSREACTIVITY STUDIES** as stated in the CurianHpSAPackage Insert.

The specificity of Curian HpSA was tested utilizing the following bacterial, fungal and viral strains. Each potentially cross-reactive microorganism was added at minimum concentrations of 1.0 x 107 CFU/ml (bacteria/fungi) or 1.0 x 105 TCID50/ml (for viruses) to a diluted, natural negative stool matrix and a contrived positive matrix sample. No crossreactivity or microbial interference with the Curian HpSA assay was observed. Adenovirus 40, *Aeromonas hydrophila, Bacillus cereus, Borrelia burgdorferi, Campylobacter coli, Campylobacter jejuni, Candida albicans, Citrobacter freundii, Clostridium difficile, Clostridium perfringens, Enterobacter cloacae, Enterococcus faecalis, E. coli* O157:H7, *E. coli, Escherichia fergusonii, Haemophilus influenzae, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa,* Rotavirus, *Salmonella spp.* Dublin, *Salmonella spp.* Hilversum, *Salmonella spp.* Minnesota, *Salmonella typhimurium* Group B, *Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus aureus* Cowan I, *Staphylococcus epidermidis, Yersinia enterocolitica.*

**Table 1 - Accuracy Testing Results**

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

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Specimen ID | Expected Result | Internal Control Line Present (Valid) | Actual Result | Performed By / Date |
| Positive Control | Positive |  |  |  |
| Negative Control | Negative |  |  |  |
| HPSA-01 | Negative |  |  |  |
| HPSA-02 | Positive |  |  |  |
| HPSA-03 | Negative |  |  |  |
| HPSA-04 | Positive |  |  |  |
| HPSA-05 | Negative |  |  |  |
| HPSA-06 | Positive |  |  |  |
| HPSA-07 | Negative |  |  |  |
| HPSA-08 | Positive |  |  |  |
| HPSA-09 | Negative |  |  |  |
| HPSA-10 | Positive |  |  |  |
| HPSA-11 | Negative |  |  |  |
| HPSA-12 | Positive |  |  |  |
| HPSA-13 | Negative |  |  |  |
| HPSA-14 | Positive |  |  |  |
| HPSA-15 | Negative |  |  |  |
| HPSA-16 | Positive |  |  |  |
| HPSA-17 | Negative |  |  |  |
| HPSA-18 | Positive |  |  |  |
| HPSA-19 | Negative |  |  |  |
| HPSA-20 | Positive |  |  |  |

**Table 2 – Accuracy testing**

**Patient Specimen Results Sheet**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Patient ID | Current Test Method Result | IC STAT Result | Internal Control Valid (Y/N) | Tech/Date | Discrepant Y/N |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

**Table 3 - Precision Validation**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Date** | **Specimen ID** | **Expected Result** | **Actual Result** | **Performed By** |
| **Day 1** |  | **HPSA-01** | Negative |  |  |
| **HPSA-02** | Positive |  |  |
| **HPSA-01** | Negative |  |  |
| **HPSA-02** | Positive |  |  |
| **Day 2** |  | **HPSA-01** | Negative |  |  |
| **HPSA-02** | Positive |  |  |
| **Day 3** |  | **HPSA-01** | Negative |  |  |
| **HPSA-02** | 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)*

*\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

*(Title*)