**Introduction**

Gastrointestinal infections in both pediatric and adult patients account for significant morbidity and mortality worldwide. Diarrheal disease can be caused by a number of pathogens including viruses, bacteria, and parasites. For detection of enteric pathogen set, conventional diagnostic procedures involve culture, microscopy, and/or stool antigen tests. A newly FDA-approved test procedure, xTAG® Gastrointestinal Pathogen Panel (GPP) which includes 15 targets (4 pending approvals), overcomes most of those shortcomings and offers an economical and efficient management for clinical patients. This study evaluates and validates the analytical and clinical performance of the GPP assay.

**Method**

Two hundred and one (201) stool specimens were collected from 140 known (+) banked samples of Luminex®, 51 clinical patients, and 10 normal volunteers. For sample preparation, 1ml of S.T.A.R. (Stool Transport and Recovery) buffer with the internal control (MS2) was added to each tube (Abbott Master Mix Tube) containing stool-collected on the swab. After mixing thoroughly, incubate the tubes at 37°C water bath for 5 minutes.  Store the tubes at −20°C until use. Thaw the tubes at 37°C water bath at least 5 minutes before centrifugation at 2000 rpm for 5 minutes. Remove any bubbles on the surface of the solution before testing procedure. Then, the samples were isolated with an open-mode protocol on Abbott’s m2000 sp automatic instrument. The GPP kits (Luminex) were used following the manufacturer’s instructions. The nucleic acid was amplified in a single multiplex RT-PCR reaction in a ProFlex thermocycler (Applied Biosystems), and the amplicon was hybridized with xMAP bead. Both the bead ID and the target reaction were detected by the Luminex®. The target-specific cut-off value was used with TDAS LSM software (xTAG® Data analysis Software LSM) to determine the presence/absence of the pathogens in the testing samples.

In addition, stored (-20°C) positive patient specimens were frozen and thawed for one to four times during a period between 2 weeks to five months to evaluate the effects of freeze/thaw cycles on the sample stability in STAR buffer.

**Results**

Each target was tested at least 6 times and all of them showed expected (+) results (Table 1 & 2). All normal stool samples were tested negative (data not shown). The external testing results were available for those 51 patients. Thirty-one out of fifty-one patients were tested negative both with GPP and outside lab (data not shown). The other 20 patient results were identified (+) targets either by GPP or outside lab (Table 3). Results of eight patients were found completely matched between two labs. There were 4 (+) results from GPP but were negative by outside lab. Another 6 (+) results were picked up by GPP but they were not ordered by physicians. Two E. histolytica dispar (+) were not detected by GPP. A total of 17 positive samples were included in the freeze/thaw study (Table 4). All specimens were stable for at least two weeks with various freeze/thaw cycles, and some could be stable up to five months. However, targets with low signals (close to cut-off value, shown in bold) did not show reproducible results after two freeze/thaw cycles. No single reaction showed the inhibitory substance.

**Conclusion**

Our study results demonstrate that GPP is superior to the conventional methods in accuracy, sensitivity, and specificity. In addition, using S.T.A.R. buffer for the isolation of stool specimens instead of pre-treating stool with the bead as recommend by Luminex streamlines the testing procedure.

**References**

1. xTAG GPP Package Insert for use with Luminex 100/200, Rev A MLD-032-KP001 Rev A. Effective Date: January 2013
2. JPCNET-6.1 Rev 2 Software User Manual
3. TDAS GPP (US) User Manual
4. TDASLSM (US) User Manual