

## Salmonellosis and xTAG Gastrointestinal Pathogen Panel (GPP)

### Introduction

Infectious gastroenteritis may be caused by a wide range of bacteria, viruses and parasites and may be difficult to differentiate from non-infectious causes. It is a major burden to health services with associated socioeconomic cost. The incidence rate of food and water borne diseases cases in Malaysia per 100,000 population is 50.33 % annually<sup>1</sup>, however the true burden of infection is probably significantly underestimated.

Rapid and accurate diagnosis of gastrointestinal infections is crucial to allow proper treatment can be initiated and appropriate infection control or epidemiologic measures can be implemented to prevent the disease from spreading. The routine laboratory diagnosis rely on a series of conventional techniques, including microscopy, culture, antigen detection and individual real-time PCR assays. These methods have demonstrated good performance, however they are labor intensive, time consuming and requiring clinicians to select appropriate tests. Multiplex molecular panels have recently become commercially available and is able to consolidate laboratory workflow, improve diagnostic accuracy and allow more efficient use of hospital resources.

### Salmonella Infection

Salmonella infection, or salmonellosis, is a common bacterial disease that affects the gastrointestinal tract. Salmonella is a group of bacteria that causes typhoid fever, food poisoning, gastroenteritis, enteric fever and other illnesses. Salmonella bacteria typically live in animal and human intestines and are shed through faeces. Humans become infected mostly through contaminated water or food.<sup>4</sup> In Malaysia, the incidence rate of salmonella infection is in its increasing trend, from 0.73% in year 2013 almost doubled to 1.42% in year 2015. The associated mortality rate is also quite alarming, which approximately 3000 people died annually in year 2015 due to typhoid fever alone.<sup>1,2,3</sup>

Salmonella infection is usually caused by eating raw or undercooked meat, poultry, eggs or egg products.

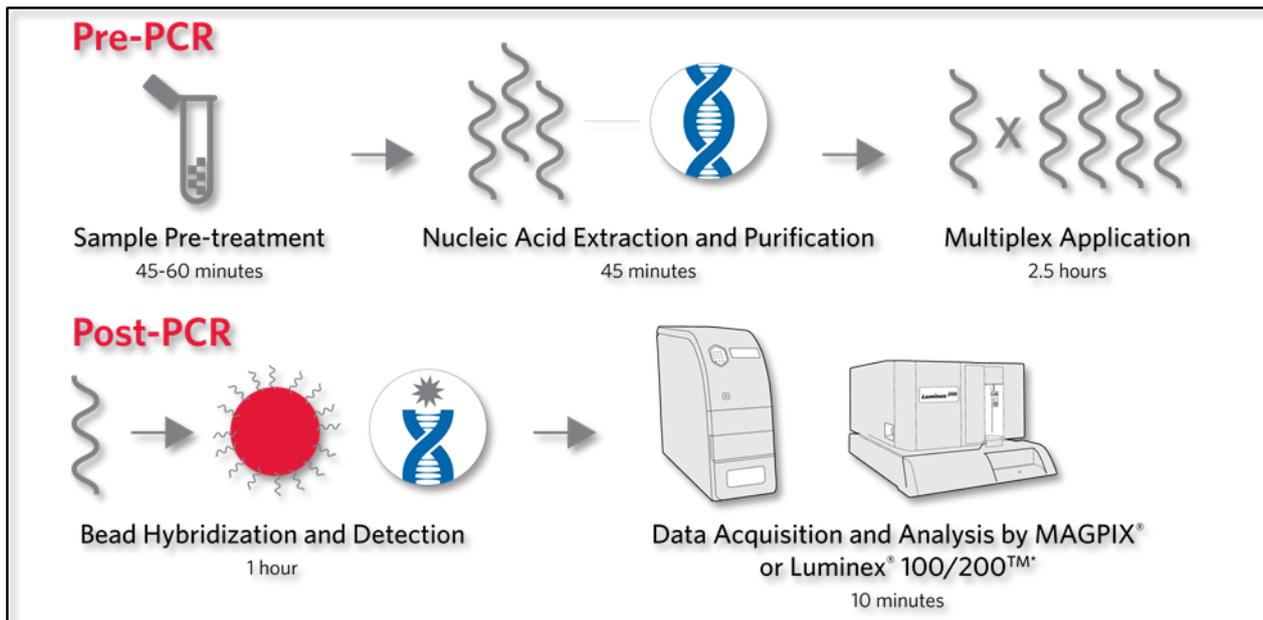
The incubation period ranges from several hours to two days. Typically, people with salmonella infection could have no symptoms. In severe cases, possible signs and symptoms developed within 8 – 72 hours include nausea, vomiting, abdominal cramps, diarrhoea, fever, chills, headache and haematochezia (blood in stool). Most healthy people recover within a few days without specific treatment. In some cases, the diarrhoea associated with salmonella infection can be dehydrating and a prompt medical attention is required. Life-threatening complications also may develop if the infection spreads to other organs beyond intestines. The risk of acquiring salmonella infection is increased for poor sanitation area.<sup>4</sup>



## Luminex xTAG® Gastrointestinal Pathogen Panel (GPP) – Diagnostic Tool for Salmonella Infection

GPP is a multiplexed molecular assay capable of simultaneously detecting adenovirus 40/41, rotavirus A, novovirus GI/GII, Salmonella spp., Campylobacter spp. (*C. jejuni*, *C. coli* and *C. lari*), Shigella spp., (*S. boydii*, *S. sonnei*, *S. flexneri* and *S. dysenteriae*), Clostridium difficile, enterotoxigenic Escherichia coli (ETEC), enterohaemorrhagic E. coli (EHEC), E. coli O157, Yersinia enterocolitica, Vibrio cholera, Giardia lamblia, Entamoeba histolytica and Cryptosporidium spp. (*C. parvum* and *C. hominis*). The assay is able to detect multiple bacterial, viral or parasitic targets from a single stool specimen.<sup>5</sup>

GPP assay is performed on 100 µl of fresh or frozen stool specimen, as well as on stool specimen collected in transport medium (i.e. Cary-Blair). Testing of 24 samples can be completed in approximately 5 hours, which involves the following steps:



- (i) sample pre-treatment,
- (ii) nucleic acid extraction,
- (iii) multiplex PCR,
- (iv) bead hybridization and
- (v) data acquisition and analysis on Magpix®/Luminex® 100/200™ analyser.<sup>5</sup>

Patel et al. evaluated the clinical performance of the GPP in intro diagnostic assay in a comparison between clinical and public health laboratories. The reproducibility study showed 98.7% sensitivity with high positive and negative agreement values (96.2% and 99.8%, respectively), while assay performance against confirmatory methods resulted in 96.4% sensitivity with similar positive and negative agreement values (90.1% and 99.5%, respectively).<sup>6</sup> There was no discrepant result found for Salmonella spp. detection using GPP as compared to the reference method used.

Another study performed by Albert et al. showing that GPP assay showed surpassed performance by detection of two-fold more pathogens as compared to the conventional assays. GPP assay has dramatically improved the diagnosis of coinfections as compared to conventional tests. The detection of coinfections varied from 7 – 38% in stool specimen by GPP assay, whereas it was either zero or negligible by conventional methods. The study proved that GPP assay manage to detect all the pathogens identified as well as additional pathogens not being detected by conventional assays.<sup>7</sup>



Vocale and colleagues also presented GPP assay managed to produce 53.61% (356/664) positive results, as compared to 45.33% by routine testing. Of the positive specimens, 34.55% demonstrated multiple pathogens detection. The routine assays were not able to identify all of the mixed infections, unlike GPP assay, which in 9/74 positive cases for *Salmonella* spp. showed the simultaneous presence of another pathogen. The detection of coinfections indicated additional investigations in order to clarify the putative role in the development of acute gastroenteritis. Detection does not necessarily indicate disease, as many pathogens can exist asymptotically (e.g. *Salmonella* spp.) or sub-clinically (e.g. *Clostridium difficile* non-toxingenic strains) in a colonization-like status.<sup>8</sup>

In conclusion, the multiplex-PCR GPP bead assay is a convenient, sensitive and consolidated assay which allows for detection of 15 major gastrointestinal pathogens or targets from a single stool specimen and may be easily incorporated in routine laboratory workflow.

## References

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