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# Verification and large scale clinical evaluation of a national standard protocol for *Salmonella* spp./*Shigella* spp. screening using real-time PCR combined with guided culture

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ABSTRACT

*Salmonella* spp./*Shigella* spp. are often associated with food poisoning and fecal-oral transmission of acute gastroenteritis that requires strict monitoring, especially among people who would handle food and water. In 2014, the National Health and Family Planning Commission of the P. R. China issued a national standard protocol (recommendatory) for the screening of *Salmonella* spp./*Shigella* spp.. However, its performance has not been fully studied. Whether it was suitable for use in our laboratory was still unknown. In the current study, the new protocol was first verified by various experiments and then its clinical performance was evaluated in about 20,000 stool samples over a three-year period. Verification results showed that the new protocol was highly specific and reproducible. Sensitivity (as defined as the lower limit of detection) of the new protocol at the PCR step was $10^3$ CFU/mL and $10^1$ CFU/mL for *Salmonella* spp. and *Shigella* spp., while that at the guided culture step was $10^4$ CFU/mL and $10^3$ CFU/mL, respectively. The large scale clinical evaluation indicated that the new protocol could increase the positivity rate by two fold and decrease the workload/median turnaround time significantly. In conclusion, the protocol was verified and evaluated and was proven to be a valuable platform for the rapid, specific, sensitive and high-throughput screening of *Salmonella* spp./*Shigella* spp.

## 1. Introduction

Despite great improvement in food/water safety and sanitary conditions, diarrhea is still a common disease among human beings with high incidence (Liu et al., 2016; Roy et al., 2006; Wilking et al., 2013). Pathogens attributed to diarrhea included various kinds of bacteria, viruses and parasites. Among them, bacteria (*Salmonella* spp./*Shigella* spp. especially) are often associated with food poisoning, fecal-oral transmission and person-to-person spread of acute gastroenteritis(Liu et al., 2016; Shen et al., 2016; Van Lint et al., 2016; Wang et al., 2010; Wikswo and Hall, 2012). Though the mortality is low and spontaneous cure occurs in a few days, some patients sustain various symptoms for weeks, which makes diarrhea one of the most serious public issues globally with very high socioeconomic impact (Friesema et al., 2012; Henson et al., 2008). Therefore, the Chinese government issued laws for obligatory *Salmonella* spp./*Shigella* spp. screening during the pre-employment physical examination among people who would handle food and water.

Routine method for the detection of *Salmonella* spp./*Shigella* spp. is culturing, which has been regarded as the gold standard. It can identify to the species level which could facilitate the disease outbreak management and antimicrobial profiling. However, it can be low in sensitivity, labor-intensive and time-consuming, which makes it unsuitable for routine testing of large numbers of samples. Moreover, interfering background flora may mask the detection and isolation of target pathogens (Kumar et al., 2008), and sometimes viable but non-culturable (VBNC) *Salmonella* spp./*Shigella* spp. may exist in stool samples (Oliver, 2005). Therefore, many laboratories have developed new techniques for the detection of diarrhea pathogens (Kamkamidze et al., 2016; Li, 2016; Mo et al., 2010; Mo et al., 2015; Qi et al., 2014; Rintala et al., 2016; Shi et al., 2006; Sun et al., 2011; Van Lint et al., 2015; Wang et al., 2016; Wohlwend et al., 2016; Zhuang et al., 2014). In October 2014, the National Health and Family Planning Commission (NHFPC) of the P. R. China issued a national standard protocol (recommendatory) for the screening of *Salmonella* spp./*Shigella* spp. among people who would handle food and water(Cheng et al., 2014).

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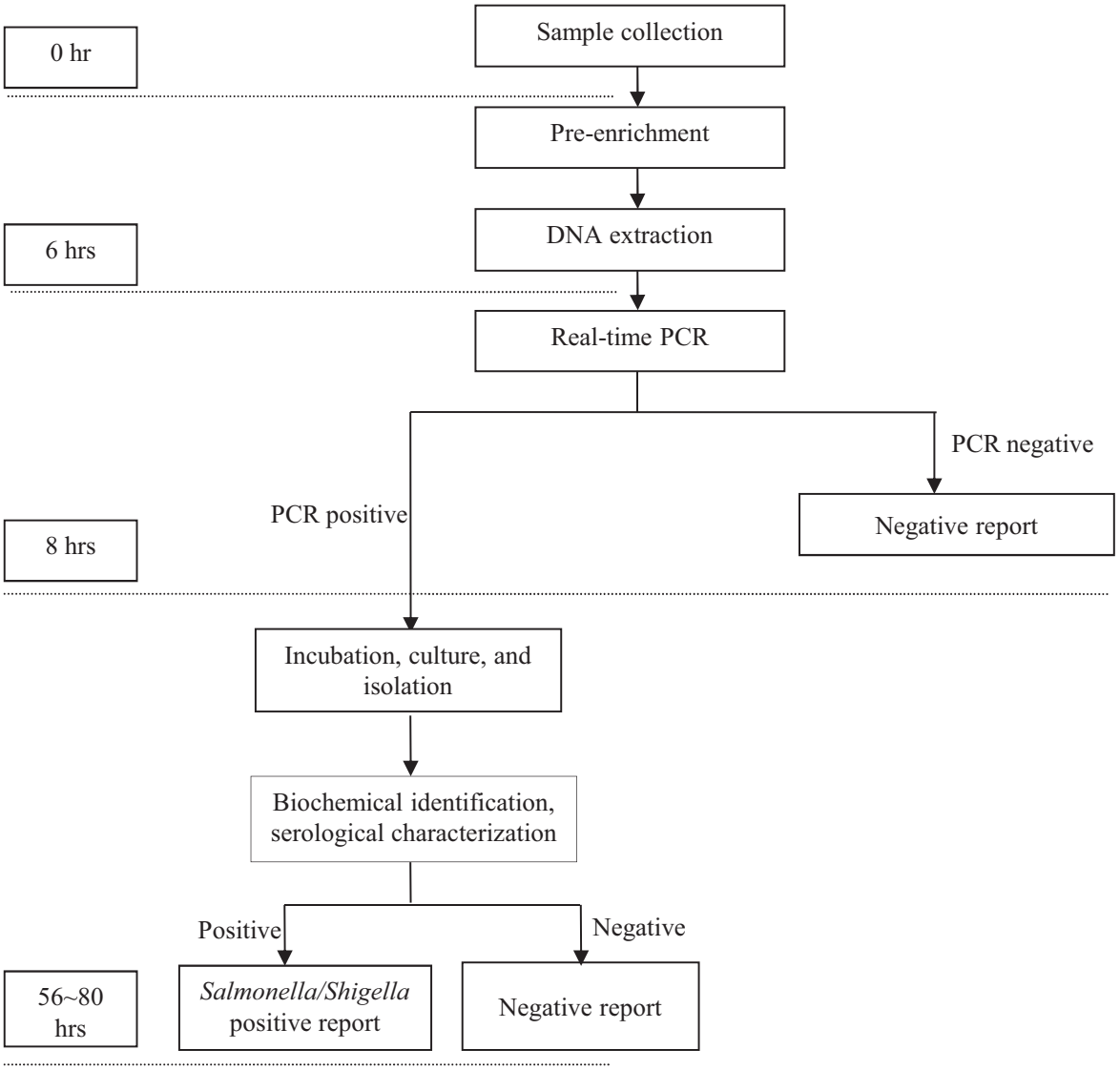


**Fig. 1.** The screening algorithm of the national standard protocol issued by the NHFPC of the P. R. China.

As shown in Fig. 1, this new protocol applied real-time PCR combined with guided culture for the detection, with the aim to increase the detection rate. However, the performance of recommendatory protocol has not been fully studied. Whether it was suitable for use in our laboratory was still unknown.

In the current study, the new protocol was first verified by conducting experiments to study its specificity, sensitivity and reproducibility. Then its clinical performance was evaluated in about 20,000 anal swab samples over a three-year period.

## 2. Material and methods

### 2.1. Clinical specimens

A total of 20,664 fresh anal swab samples were collected from people who would handle food and water between 2014 and 2016. In 2014, anal swab samples (n = 6089) were detected by conventional culture, while in 2015 and 2016, anal swab samples (n = 8269 and 6306, separately) were detected by real-time PCR combined with guided culture (the new protocol). The study was conducted in accordance with the ethical standards set by the ethical review committee of Zhuhai International Travel Healthcare Center and with the 2013 Helsinki declaration. Informed consent was obtained from each study participant.

### 2.2. Real-time PCR assay

A duplex real-time PCR assay was used to detect *Salmonella* spp. and *Shigella* spp. simultaneously. Briefly, anal swab samples were subjected to a 6 h' pre-enrichment in 3 mL Nutrient Broth (LandBridge, Beijing, P. R. China) at 36 °C and then 1 mL of the pre-enrichment culture was centrifuged at 800 rpm for 2 min to allow large particles to settle down, and then the supernatant was centrifuged at 12000 rpm for 5 min. DNA was extracted from pellet by boiling at 100 °C for 5 min in 100 uL DNA extraction solution (0.01 M pH 8.0 Tris-EDTA, 0.01% NP40). The primers and probes used (Table 1) were synthesized by Life Technologies (Guangzhou, P. R. China) (Shi et al., 2006). The reaction mixture contained 12.5 μL 2 × Premix PrimeSTAR HS (Takara, Dalian, P. R. China), 0.4 μM of each primers, 0.2 μM of each probes, and 5 μL template. The cycling program was 95 °C 3 min, followed by 40 cycles of 95 °C 15 s, 55 °C 30s, 72 °C 34 s. Fluorescent signals were collected from FAM and HEX channels at the elongation step. As PCR inhibitor may appear in the anal swab samples which could interfere with the detection of *Salmonella* spp./*Shigella* spp., approximately 25 ng of mouse genomic DNA (Promega Corporation, Madison, WI) was spiked into the sample as internal control to check the efficiency of removal of