Salmonella detection from faecal samples and food products by using a novel, fast and specific isothermal amplification technology, SIBA®

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BACKGROUND

Salmonellosis, caused by *Salmonella enterica* subsp. *enterica*, is one of the most commonly and widely distributed foodborne diseases. *Salmonella* carrying animals and humans cause considerable problems in primary production and food processing industry. We developed a fast and accurate nucleic acid based detection system for *Salmonella* carriers with very low amounts of bacteria in their faeces as an alternative to the widely used and time-consuming culture based method.

METHODS

We used a selective selenite broth for enrichment of *Salmonella* from human faecal samples and buffered peptone water (BPW) for enrichment of *Salmonella* from animal faeces and dairy products. We also tested the compatibility of Rappaport-Vassiliadis-Soypeptone (RVS), broth widely used for enrichment of environmental and food product samples, with our test system. Moreover, we analysed 51 clinical *Salmonella* positive carrier samples (in Transpocult gel tubes) with our test system and compared the results to an IVD-marked PCR based acute Salmonella test and to a published Salmonella PCR test[2].

The isothermal DNA amplification method, Strand Invasion Based Amplification (SIBA®) [1], was used for the detection of the bacteria in faeces. SIBA is based on sequence specific recombinase activity allowing hybridization of primers, which are then extended by DNA polymerase (Figure 1). In the SIBA based *Salmonella* assay, the reaction mix is freeze-dried and rehydrated at use. Reactions are detected with a novel IO Quenching chemistry, which provides a sequence specific, fluorophore and quencher based probeless detection method. The assay contains an internal control in multiplexed reactions.

The workflow (Figure 2) includes enrichment incubation and sample preparation, where 200 µl of enriched growth medium is transferred into a lysis reagent. After a filtration step, the sample is heated, mixed with reaction buffer and added to a tube containing lyophilised reagents. The assay is run on the Orion GenRead®, a self-contained, HIS/LIS compatible and portable instrument which needs no external PC or other extra devices to be operated. The detection is carried out with fluorescent readout together with an integrated qualitative algorithm and an internal amplification control (example of amplification curves shown in figure 3). The sample preparation takes only around 10 minutes, followed by fast amplification of 50 min or less.

Figure 1. Oligonucleotides in Strand Invasion Based Amplification (SIBA®) assay

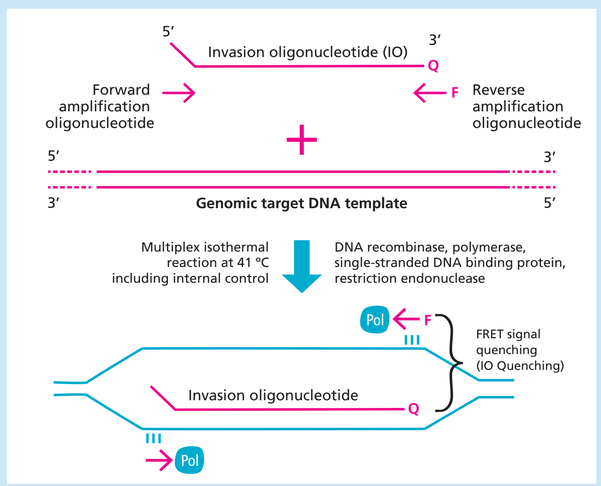


Figure 2. Salmonella assay workflow with different sample types

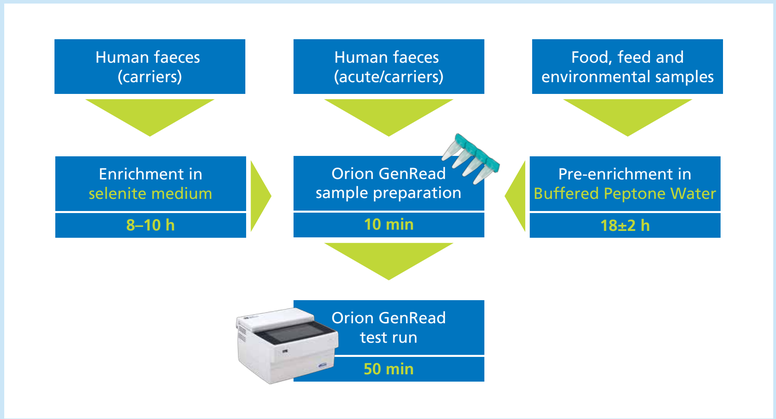
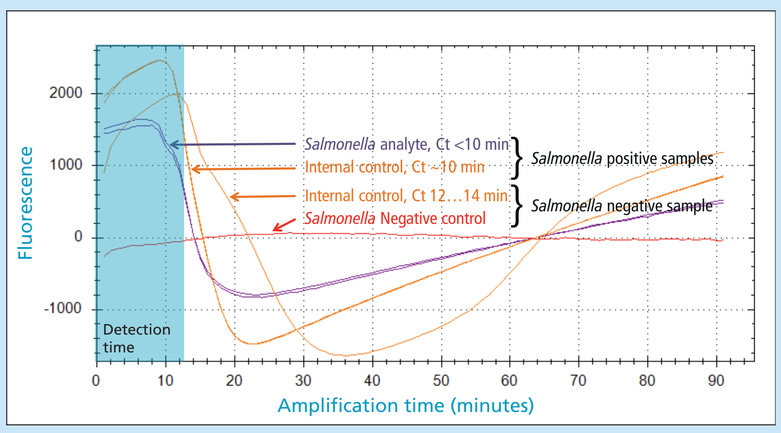


Figure 3. Example of Salmonella assay amplification curves



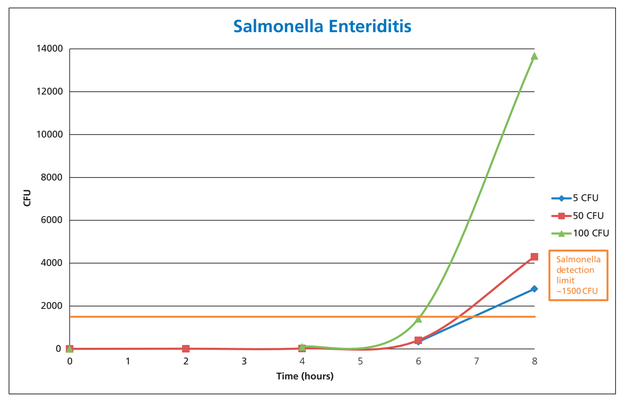
RESULTS

The doubling time of *Salmonella* in selenite medium was determined with and without human faeces. The doubling time without faeces was found to be 37 minutes (growth curves shown in Figure 4). With faeces, our test was able to detect down to 1 CFU of *Salmonella* (as a starting material) after only 8 hours of incubation in selenite medium.

With milk as well as cattle faeces, our test was able to reliably detect only 1 CFU of *Salmonella* (as a starting material) after 16 hours of incubation in BPW. We also found RVS broth compatible with our test system. The sample materials tested with our test system so far include human faeces, cow´s faeces, horse faeces, and milk.

Among the clinical *Salmonella* carrier samples tested without the enrichment step, 48/51 (94%) were found positive with SIBA based *Salmonella* assay, 46/51 (90%) with the commercial PCR test and 33/51 (65%) with the published PCR test.

Figure 4. Growth curves of *Salmonella Enteriditis* in selenite medium with 5, 50 and 100 CFU as a starting material



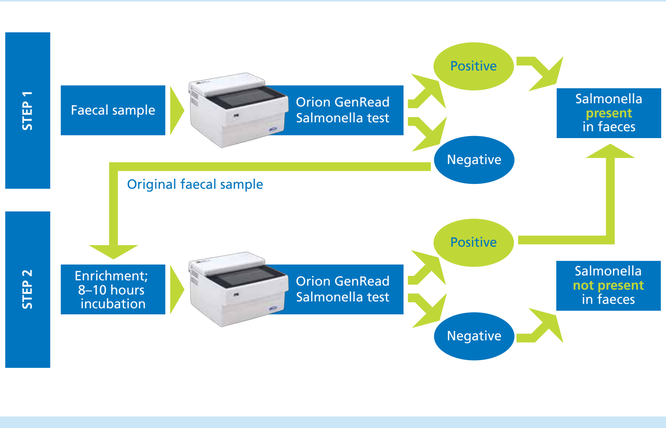
CONCLUSIONS

According to our results, SIBA based *Salmonella* assay provides fast *Salmonella* human carrier screening within 10–12 hours at maximum (including 8–10 hours incubation, sample preparation and test run).

Based on the clinical sample data, the SIBA based *Salmonella* assay could serve in practice as a simple two-step procedure including (1) testing directly from the faecal sample and (2) in the case of negative result, enrichment incubation and re-test (Figure 5). With minor modifications, this system will also be suitable for testing environmental samples and food products, among others.

Accelerating the *Salmonella* carrier screening offers cost saving opportunities e.g. for industrial employers who need to hold employees suspected to carry *Salmonella* from work. In these situations faster testing could save working days. For breeders with legally defined animal testing, shortening testing time is equally valuable. In addition, the portability and battery operation enable the Orion GenRead instrument to be taken on-site for instance in the case of local *Salmonella* epidemics.

Figure 5. The prospective 2-step procedure of Salmonella carrier samples



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1. Hoser, M.J., Mansukoski, H.K., Morrical, S.W. and Eboigbodin, K.E. (2014). Strand Invasion Based Amplification (SIBA®): A Novel Isothermal DNA Amplification Technology Demonstrating High Specificity and Sensitivity for a Single Molecule of Target Analyte. *PLoS One*, **9**(11).
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