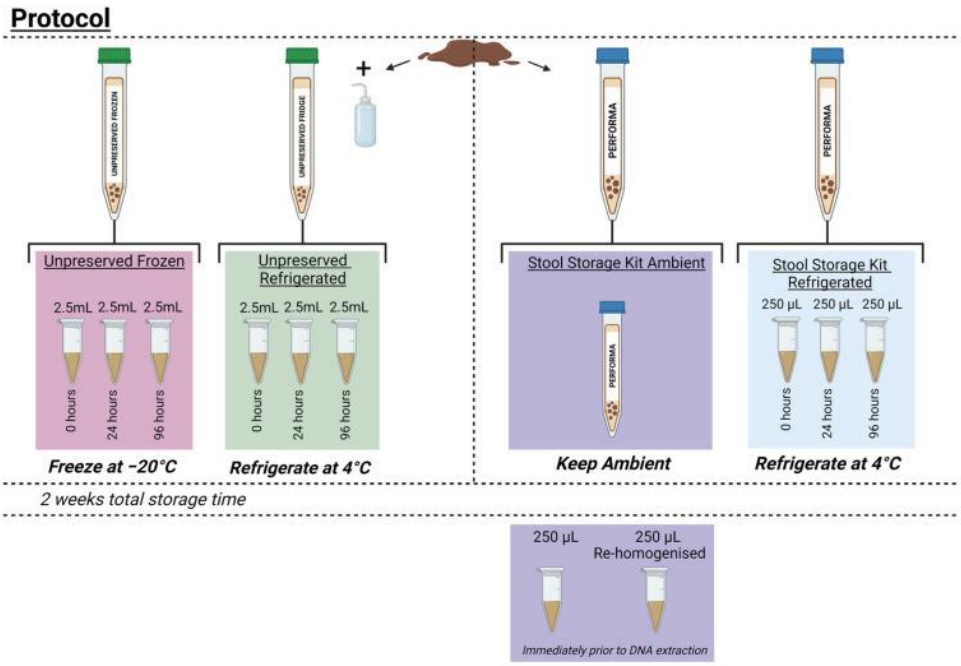
Identification of a Sub-Clinical *Salmonella* spp. Infection in a Dairy Cow Using a Commercially Available Stool Storage Kit

Sampling stools from animals is a useful way of diagnosing diseases. Faeces contain large numbers of micro-organisms, including bacteria, and, to preserve these, farmers and owners are advised to store the stool samples in a fridge at 4 °C. However, in a farm setting, access to working onsite refrigeration is not guaranteed. This means that samples can often sit at ambient temperature for several days until they reach the laboratory for analysis, and these may be located at significant distances from the farms. Leaving faeces at room temperature can lead to a significant change in bacterial composition, which can result in bacterial overgrowth and difficulty in detecting certain species for diagnosis, such as *Salmonella* species. The sub-clinical disease burden of *Salmonella* spp. in cattle can become significant for farmers. However, current methods of faecal sampling in a rural setting for diagnosis are not consistently sufficient for the preservation of *Salmonella* spp. in faeces. Therefore, this study evaluated the use of a commercial stool storage kit, the PERFORMAbiome.GUT (DNA Genotek), for bacterial preservation in cow faecal samples compared to unpreserved stools placed into refrigeration at different time-points. A stool sample was collected per-rectum from one apparently healthy Holstein–Freisen cow. The sample was weighed and aliquoted into two sterile Falcon tubes and into two commercial stool storage kit tubes. After homogenisation, both Falcon tubes containing unpreserved stool, and one commercial kit tube were further aliquoted into microcentrifuge tubes and then placed into refrigeration at 4 °C at 0, 24, and 96-hours after processing. Stool in the remaining commercial storage kit was not aliquoted and remained in the tube at ambient temperature, as per manufacturer recommendations.

After 2-weeks, DNA was extracted from the samples and analysed using endpoint PCR, revealing a sub-clinical infection with *Salmonella* spp. The bacterium was best preserved when the stool was stored in the commercial kit at ambient temperature and re-homogenised immediately prior to DNA extraction; *Salmonella* spp. DNA was detectable in the faeces after 2-weeks. However, the unpreserved stool did not maintain obvious levels of *Salmonella* spp. after 24-hours at ambient temperature.

The burden of sub-clinical salmonellosis in cattle should not be understated, as demonstrated by the incidental finding of a sub-clinical infection in this study. The use of the preservative commercial kits should be given consideration by veterinary practices and clients for inclusion in routine annual herd health checks. However, to make these recommendations, the next steps should include using the commercial kit to collect faeces from a larger number of animals and sampling a whole herd of apparently healthy dairy cows in a farm setting to detect levels of sub-clinical salmonellosis. Further exploration of the performance of the stool storage kit for longer-term storage of stool would also be useful. The use of bacterial culture on unpreserved stool to serve as a comparison to PCR and qPCR techniques should be considered for future experiments, as well as pathogen-spiking studies to explore the impact of a changing microbiome on PCR and qPCR analysis.


Protocol
UNPRESERVED FROZEN
UNPRESERVED FRIDGE
PERFORMA
PERFORMA
Unpreserved Frozen
2.5mL 2.5mL 2.5mL
0 hours
24 hours
96 hours
Freeze at −20°C
Unpreserved Refrigerated
2.5mL 2.5mL 2.5mL
0 hours
24 hours
96 hours
Refrigerate at 4°C
Stool Storage Kit Ambient
PERFORMA
Keep Ambient
Stool Storage Kit Refrigerated
250 µL 250 µL 250 µL
0 hours
24 hours
96 hours
Refrigerate at 4°C
2 weeks total storage time
250 µL
250 µL
Re-homogenised
Immediately prior to DNA extraction