SUPPLEMENTAL MATERIAL

PREPARATION OF DRIED STOOL SPOTS ON FILTER PAPER AND FROZEN STOOL METHODS

For the frozen stool (FS) method, a total of 70 mg from the fecal samples were filled into cryovials and directly stored at −20°C as described previously.[1,2]

For the dried stool spots on filter paper (DSSFP) method, firstly, 70 mg from the stool samples were diluted with 300 μL of saline. After homogenization, the samples were spread onto the ordinary filter papers (which are used in laboratories for general purposes such as basic filtration and sample preparation) as thin layer with a diameter of approximately 3 cm. After air-dried, the filter papers containing the stool spots were stored individually in sealed polyethylene bags at room temperature until the DNA extraction procedure.[3]

Additionally, five samples, which were negative for polymerase chain reaction (PCR) and microscopy, were kindly provided by the National Reference Laboratory and all procedures were also applied for these negative control samples.

DNA EXTRACTION FROM DSSFP AND FS SAMPLES

Maximum duration of storage was 3 months for both the methods. DNA extraction was conducted according to the manufacturer's protocol (EURx, GeneMATRIX Stool DNA Purification Kit, Gdansk, Poland). A total of 70 mg from frozen fecal samples were used for the extraction of DNA with a final elution volume of 100 μL. The kit's procedure was based on bead-beating methods, while horizontal vortexing at the highest speed was preferred for achieving the optimal DNA yield.[3]

Dried stool samples on the filter papers were scraped very carefully under a biosafety cabinet Class II, and disposable materials were used for each sample to avoid contamination of DNA between the specimens and infection risk for the researchers. Powdery stool particles collected from the scrapings were extracted by using the same DNA extraction kit with a final DNA elution volume of 100 μL. For the control of possible presence of DNA on the filter papers, the extraction protocol (EURx Stool DNA Extraction Kit) was also applied to an additional filter paper that did not contain any stool sample.[3]

EVALUATION OF DNA CONCENTRATION, PURITY, AND PCR INHIBITORS

DNA yield was measured by Thermo Scientific™ NanoDrop™ 2000 Spectrophotometers (Waltham, MA). One microliter from the extracted DNA was evaluated in duplicate. Control of possible PCR inhibitors was done according to the method of Stensvold and others (2006).[4] Briefly, 1 μL from PCR-positive DNA and 4 μL from PCR-negative DNA were mixed up and the standard PCR was evaluated for the presence of expected amplicons.

SUPPLEMENTAL REFERENCES

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