Supporting Information

Microparticles as Viral RNA Carriers from Stool for Stable and Sensitive Surveillance

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The Difference Between the Two Protocols

The current method has significantly reduced the hands-on steps by cutting down the number of sample processing steps to three, which are namely; a) viral lysis b) PIN-cDNA pool synthesis, and c) qPCR. Compared to the spin-mini methods, RNA preparation alone has about six steps which include several tube changes, vortex mixing, and centrifuging steps. In general, both methods require a minimum of 30 min to accomplish either PIN-cDNA pool generation or RNA purification steps.

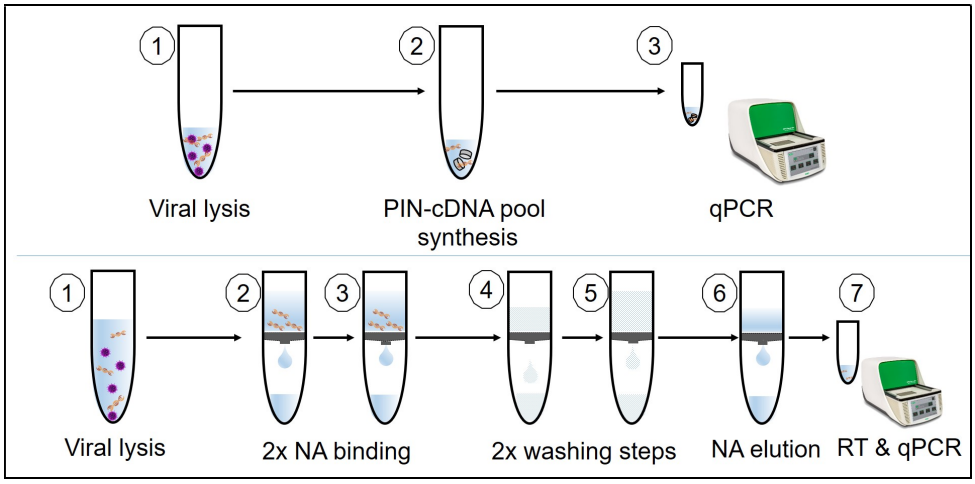

Figure S1. Schematic presentation of the differences between the two protocols. The PIN-cDNA pool qPCR protocol (top), and Spin-column RNA preparation (bottom).

The Stool Samples Contain PCR Inhibitors

One-step RT-qPCR was completely inhibited when synthetic RNA spiked with negative stool samples (1g/10 mL) was analyzed without heat intervention (n=4). Similarly, qPCR was inhibited as synthetic DNA was spiked into a similar matrix. The findings confirmed the presence of active PCR inhibitors in this matrix, with a drastic inhibitory effect on the amplification process as previously reported [1, 2]. The introduction of heat treatment adopted for viral lysis had profound results in suppressing the effect of heat-labile PCR inhibitors. Heat treatment at 100 °C for 10 min was sufficient to overcome the inhibition

effects to obtain clear amplification signals. The DNA sample was 100-fold diluted (red curve) in reference to DNA only as control (black curve) without dilution.

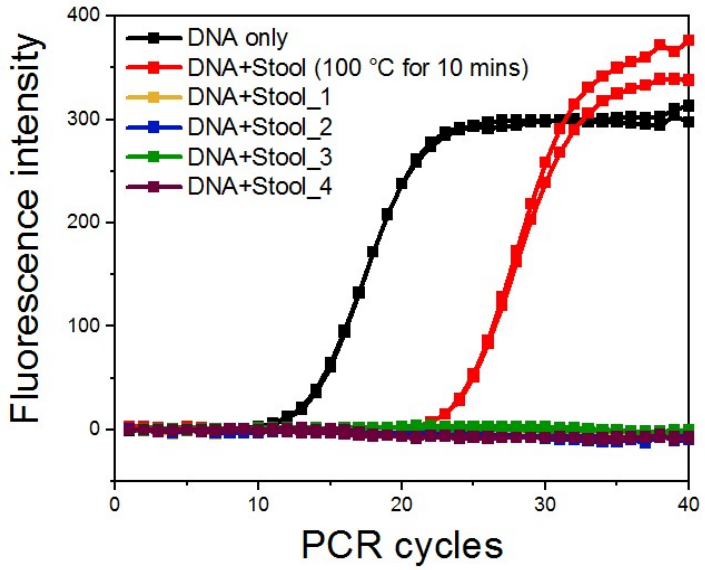

Figure S2. Complete qPCR inhibition of pure DNA spiked in a stool sample (1g/10 mL) without any pre-treatment procedure. The aqueous phase qPCR was conducted in duplicate using ultra-fast real-time PCR (G2-4) System.

Viral RNA Detection Against High Stool Concentration

Stool samples are generally suspended in an aqueous buffer at the concentration of 1g/10 mL (10%, w/v) as standard. We were able to obtain clear amplification signals using PIN-cDNA pool assay with thicker stool suspension of 5g/10 mL of PBS buffer. Although the assay sensitivity was preferable with 1g/10 mL stool suspension, the ability to capture viral RNA and synthesize PIN-cDNA pools against thicker and non-centrifuged stool suspensions is an added advantage to the current method.

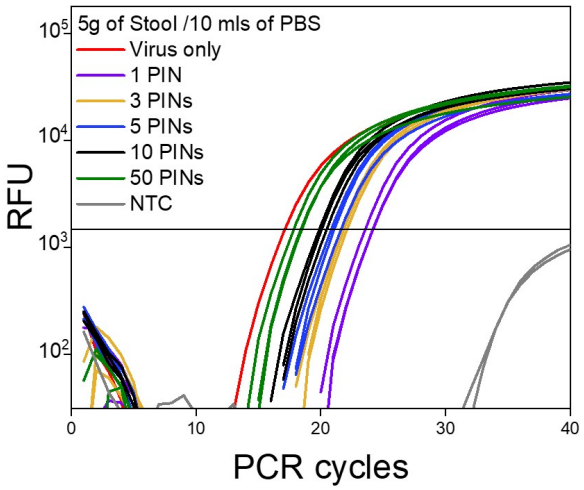

Figure S3. The log amplification curve for 5g/10 mL of stool samples against the number of PIN particles used (n=15).

The Prototype Heating Device

The compact heater, a newly designed 12V portable thermostat device with dual plates, provides room for concurrent heat treatment for viral lysis and PIN-cDNA pool synthesis. The device was used in parallel with validated commercially available dry heating blocks.

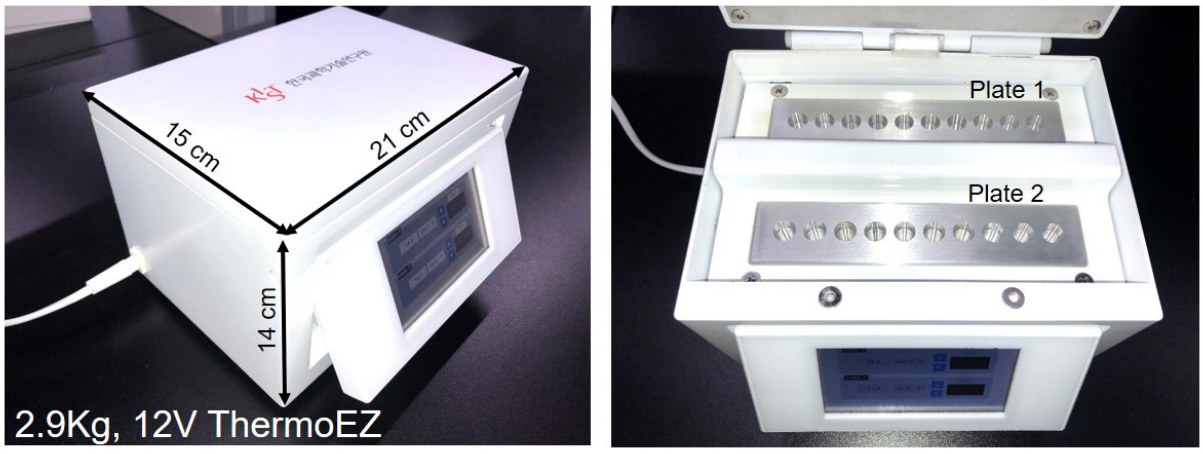

Figure S4. A prototype heating device with 10 sample wells in each plate. Plate 1 for heat treatment at 100 °C; Plate 2 for RT step at 42 °C.

References

1. Das, A.; Spackman, E.; Pantin-Jackwood, M. J.; Suarez, D. L., Removal of real-time reverse transcription polymerase chain reaction (RT-PCR) inhibitors associated with cloacal swab samples and tissues for improved diagnosis of Avian influenza virus by RT-PCR. *Journal of Veterinary Diagnostic Investigation* **2009,** *21* (6), 771-778.

2. Nechvatal, J. M.; Ram, J. L.; Basson, M. D.; Namprachan, P.; Niec, S. R.; Badsha, K. Z.; Matherly, L. H.; Majumdar, A. P.; Kato, I., Fecal collection, ambient preservation, and DNA extraction for PCR amplification of bacterial and human markers from human feces. *J Microbiol Methods* **2008,** *72* (2), 124-32.