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Supplementary Material of Manuscript

High-throughput malaria parasite separation using a viscoelastic fluid for ultrasensitive PCR detection

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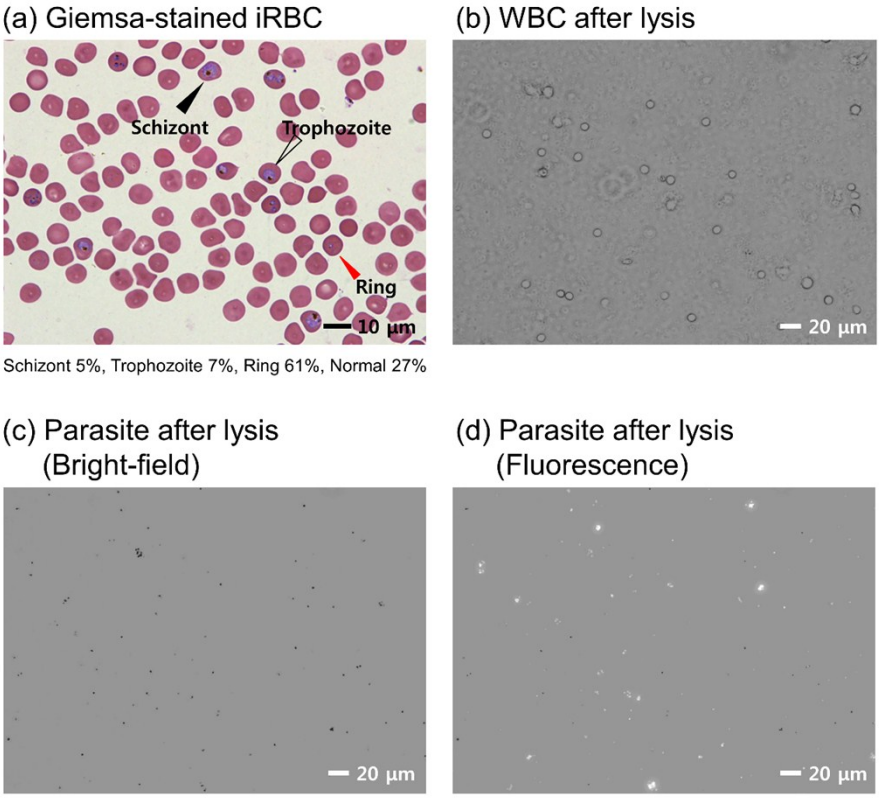

Figure S1. Microscope images of the biological samples (a) Giemsa-stained malaria infected red blood cells (iRBCs) before lysis. The sample contains iRBCs at various developmental stages. Red, empty-black and solid-black triangles indicate typical ring, trophozoite and schizont stages respectively. Rings have cytoplasm and one- or two chromatic dots. Older ring stage parasites are referred to as trophozoites and have denser cytoplasms. Schizonts contain divided nuclei and dark malaria pigments. Our sample was composed of 27% of normal RBCs, 61% of rings, 7% of trophozoites and 5% of schizonts. (b) White blood cells (WBCs) after RBC lysis. WBCs diameters were measured to be 12 $\pm$ 3 μm. (c) Bright-field microscopy image and (d) fluorescence microscopy image of Plasmodium falciparum parasites stained with Acridine Orange (AO) after lysis. AO dye stains the nucleic acids of the malaria parasites, enabling debris after lysis to be distinguished from the parasites by comparing the bright-field and the fluorescence microscopy images. The empty-white triangles in (c) and (d) indicate the same stained parasites under the bright-field and the fluorescence microscopy. From (c) and (d), the size of the malaria parasites was measured to be 1.8 $\pm$ 0.7 μm using the image analysis software ImageJ. According to the size distribution of both malaria parasites and WBCs, 2- and 10-μm particles were chosen to serve as analogues to the malaria parasites and WBCs respectively. Malaria parasites and WBCs can be successfully separated based on their size difference, considering that the elastic force (F_E) exerted on suspended cells is dependent on the particle size ($F_E \sim a^3$).

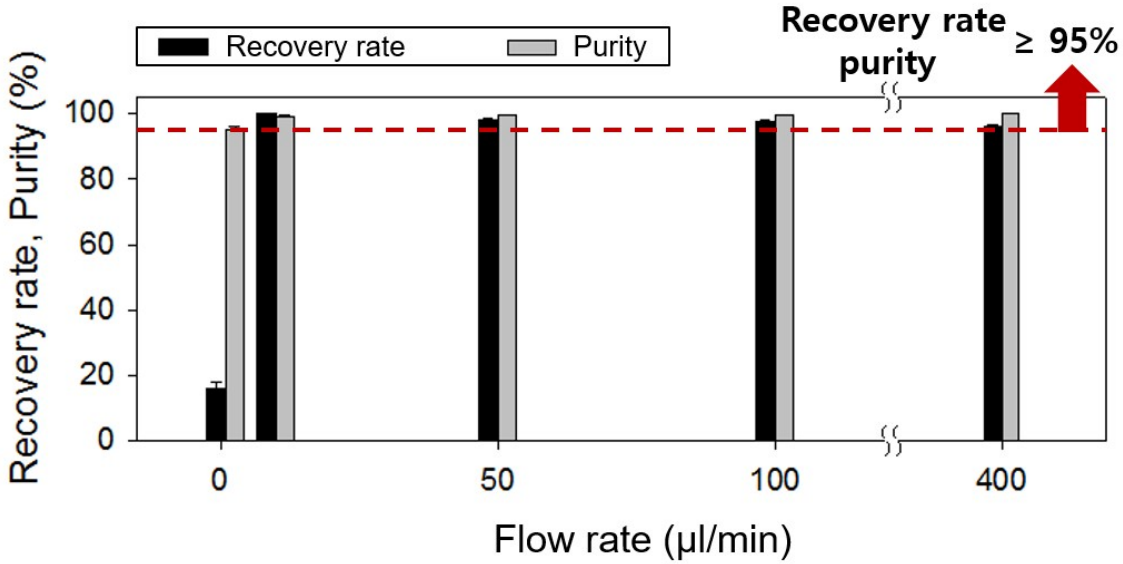

Figure S2. Effect of flow rate on recovery rate and purity of 2-μm particles at Q = 1, 10, 50, 100 and 400 μl/min. At Q = 1 μl/min, the recovery rate of 2-μm particles was ~16 % due to poor focusing in the 1st stage and random dispersion in the 2nd stage. At $Q \geq 10$ μl/min, the recovery rate rapidly increased from ~16% to higher than 95% owing to enhanced center-focusing in the 1st stage of the microchannel. In addition, the purity of separated 2-μm particles increased with increasing flow rates, because the lateral migration of 10-μm particles toward the center was enhanced at higher flow rates.