



ESI, Figure 1: (a) Schematic representation of the protocol for implementing the RPA assay on AM-EWOD device. An *E.coli* suspension is prepared (1), from which DNA containing *bla*_{CTX-M-15} (2) is extracted. DNA sample, RPA reaction mix, magnesium acetate and no template control (NTC) is pipetted onto array elements (3). Daughter droplets are dispensed from the reagent droplets (4). DNA and NTC is mixed with dispensed RPA reaction mix daughter droplets (5) and DNA-RPA reaction mix droplets are moved to the fluorescence detection position on EWOD (6). Magnesium acetate is mixed with the DNA-RPA reaction mix droplets to initiate the RPA reaction and the device heated to 39°C (7). Real-time fluorescence is measured using custom-made optical setup, while continuously mixing the reaction droplets.

Reagent	Droplet volume programmed (nL)	Average volume dispensed (nL)	Standard deviation	CV (%)	Error in dispensing (%)
Water	45	46.55	0.094	1.01	3.4
	80	84.1	0.29	1.73	5.1
	125	114.72	0.31	1.37	8
	180	168.15	0.194	0.57	6.5
Magnesium acetate	45	44.9	0.082	0.91	0.2
	80	82.95	0.089	0.53	3.6
	125	121	0.106	0.44	3.2
	180	176	1.26	3.59	2.2
RPA reaction mix	45	46.5	0.08	0.92	3.3
	80	77	0.17	1.15	3.7
	125	120.7	0.52	2.17	3.4
	180	167.6	0.786	2.34	6.8

ESI, Table 1: Error (%) and CV (%) in dispensing 15 daughter droplets from a reservoir pad for a given programmed volume and reagent (droplets removed and then returned to the reservoir). All the reagents (water, magnesium acetate and RPA reaction mix) contain 0.1% Tween® 20.