

| Sample | MERS-CoV ORF1A RNA | Bst 2.0 | AMV RT | Betaine |
|--------|----------------------------|---------|---------|---------|
| 1 | 9 x 10 ⁸ copies | 8 units | 2 units | 0.4 M |
| 2 | 9 x 10 ⁸ copies | 8 units | 2 units | 1 M |
| 3 | 9 x 10 ⁸ copies | 8 units | 0 | 0.4 M |
| 4 | 9 x 10 ⁸ copies | 8 units | 0 | 1 M |
| 5 | 0 | 8 units | 2 units | 1 M |
| 6 | 0 | 8 units | 2 units | 0.4 M |

Supplementary Figure S4. Optimization of one-pot RT-LAMP assay for RNA detection using *in vitro* transcribed MERS-CoV target RNA. The primer set ORF1a.55 was used for optimizing the RT-LAMP-mediated amplification of 9 x 10⁸ copies of DNase I-treated synthetic ORF1a RNA. RT-LAMP reactions were performed at 65 °C with 3 min incubations per cycle on a LightCycler 96 real-time PCR machine. Amplicon accumulation was measured in real-time as increase in fluorescence of the intercalating dye EvaGreen.