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Supporting Information

Amplicon Competition Enables End-Point Quantitation of Nucleic Acids Following Isothermal Amplification

Yu Sherry Jiang,[a, b] Apollo Stacy,[a] Marvin Whiteley,[a] Andrew D. Ellington,[a] and Sanchita Bhadra*[a]

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Supplementary Text T1

Theoretical framework of thresholded LAMP-OSD

In a LAMP-OSD reaction the maximum possible fluorescence amplitude (F_{max}) is determined by the maximum amount of OSD-compatible LAMP amplicon loops (L_{max}) generated. This relationship can be expressed as:

$$F_{max} = L_{max} \text{ ------ (1)}$$

Since L_{max} is transduced into F_{max} via the strand displacement probes, the observed fluorescence (F) also depends on the amount of reporter (OSD) added to the system. Furthermore, in a thresholded LAMP-OSD system, L_{max} may include both true (L_T) and false (L_F) LAMP amplicon loops. Since OSD fluorescence is triggered only by L_T:

$$F = \min(OSD, L_T) \text{ --------- (2)}$$

In the presence of L_F the amount of L_T will be attenuated. Let us assume that β is the attenuation factor. Since T_F is designed to have the same amplification kinetics as T_T we assume that:

$$\beta = \frac{T_F}{T_T} = \frac{L_F}{L_T} \text{ --------- (3)}$$

Note that β is only defined when $T_T \neq 0, L_T \neq 0$ and $\beta \geq 0$

$$L_T = \frac{T_T}{(T_T + T_F)} L_{max}$$

$$\therefore L_T = \frac{1}{(1 + \left(\frac{T_F}{T_T}\right)} L_{max}$$

$$\therefore L_T = \frac{1}{(1+\beta)} L_{max} \text{ ------- (4)}$$

Using equation (1), equation (4) transforms to:

$$\therefore L_T = \frac{1}{(1+\beta)} F_{max} \text{-------(5)}$$

Substituting equation (5) in equation (2) we get:

$$F_\beta = \min\left(OSD, \frac{1}{(1+\beta)} F_{max}\right) \text{------ (6)}$$

Where F_β denotes F at a certain value of β.

When $L_F = 0$ then $\beta = 0$ and $L_T = L_{max}$.

$$\therefore F_{\beta=0} = \min(OSD, F_{max}) \text{------ (7)}$$

The analysis beyond this point addresses the effect of reporter availability on F_β. We will consider the mutually exclusive situations of either saturating reporters or limiting reporters.

Case A: Let us first examine the effect of saturating reporters where,

$$OSD \geq L_{max} \text{ ----- (8)}$$

Note that equation (8) and $\beta \geq 0$ imply that:

$$OSD \geq \frac{1}{(1+\beta)} L_{max} \text{-------- (9)}$$

Using equations (1), (8) and (9), equation (6) reduces to:

$$F_\beta = \frac{1}{(1+\beta)} F_{max} \text{-------(10)}$$

$$Or,\ \beta = \left(\frac{F_{max}}{F_\beta}\right) - 1 \text{---- (11)}$$

Case A signifies that OSD sufficiency enables detection of all available L_T, such that fluorescence signal is determined solely by the amount of L_T. Therefore, any attenuation (β) in L_T caused by the presence of L_F will be evident as an observable decline in F_β when compared to F_{max} (see **Supplementary simulation 1**).

Case B: In practice OSD saturation may not be practical due to two reasons: First, though the maximum amount of LAMP amplicons generated is determined by the amount of primers added, L_{max} is unpredictable due to the inherent structural complexity of LAMP amplicons. Second, unlimited OSD cannot be practically added to a LAMP reaction because at very high concentrations OSD reporters often decelerate LAMP and introduce variability in fluorescence amplitude (**Supplementary Figure S1**).

Therefore, let us examine the situation of limiting reporters where $OSD < L_{max}$. This situation signifies that since the amount of reporters is limited, all available L_T may not be transduced into fluorescence. This in turn implies that depending on the magnitude of β its impact on L_T may or may not be detectable. Let us consider either outcome separately:

Case B.1. If β does not reduce L_T to levels below that of OSD such that:

$$OSD < \frac{1}{(1+\beta)} F_{max} < F_{max}$$

Then equations (6) and (7) reduce to:

$$F_\beta = F_{\beta=0} = OSD \text{------ (12)}$$

Case B.1 implies that if β is not high enough to attenuate L_T to levels below that of *OSD* then β will have no impact on F_β and hence will not be measurable (see **Supplementary simulation 1**).

Case B.2. If β reduces L_T to levels below that of OSD such that:

$$\frac{1}{(1+\beta)} F_{max} < OSD < F_{max}$$

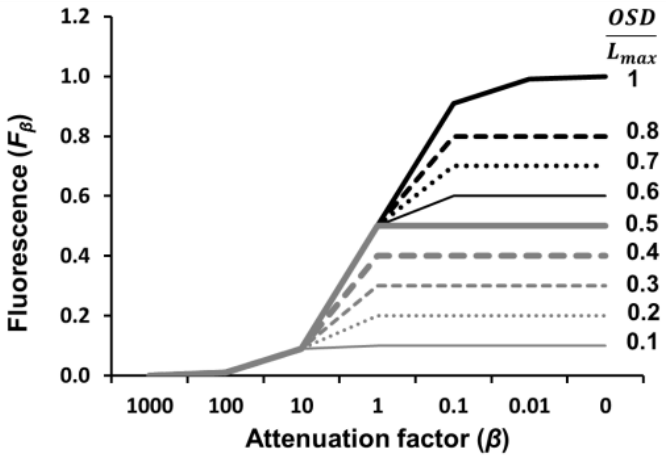
Then equation (6) reduces to:

$$F_\beta = \frac{1}{(1+\beta)} F_{max}$$

$$Or,\ \beta = \left(\frac{F_{max}}{F_\beta}\right) - 1 \text{-------- (11)}$$

Case B.2 implies that even in the absence of saturating OSD if β is large enough to reduce L_T to levels below that of OSD it will result in a detectable decline in F_β thus allowing β measurement

(see **Supplementary simulation 1**). It should be evident that in an experimental scenario since T_F is a known quantity, establishment of β will allow calculation of T_T.



Supplementary simulation 1. Thresholded LAMP-OSD simulations depicting the effect of signal attenuation (β) and OSD saturation (OSD/L_{max}) on observed fluorescence (F_β) when F_{max} is assumed to be 1.

Supplementary Text T2

Materials and methods

Chemicals and Oligonucleotides. All chemicals were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.) unless otherwise indicated. All enzymes and related buffers were purchased from New England Biolabs (NEB, Ipswich, MA, U.S.A.). All oligonucleotides were obtained from Integrated DNA Technologies (IDT, Coralville, IA, U.S.A.). Oligonucleotide sequences are summarized in **Supplementary Table T1**.

False target design. False targets were created by randomizing the OSD-binding LAMP loop sequence of the true target using 5 cycles of randomization with a web-based tool accessed from http://www.cellbiol.com/scripts/randomizer/dna_protein_sequence_randomizer.php. This randomized sequence was then substituted into the corresponding position of the target sequence by overlap PCR using mutagenic primers harboring the randomized sequences. The target and false target sequences used in this study are detailed in **Supplementary Table T2**.

Plasmid preparation. Target sequences for MERS1B, HF183 and FN1868 were purchased as gene blocks from IDT. A T7 RNA polymerase promoter was included upstream of the FN1868 target to enable RNA transcription. The target sequences for NRP2 and BRAF were built by overlap PCR using Phusion DNA polymerase (New England Biolabs (NEB), Ipswich, MA, USA). The gene blocks and agarose gel-purified PCR products were cloned into pCR2.1TOPO plasmid (Thermo Fisher, Waltham, MA, USA) by Gibson assembly using the 2X Gibson master mix from NEB and the manufacturer's protocol. All plasmids used in this study were verified by sequencing at the Institute of Cellular and Molecular Biology Core DNA Sequencing Facility.

Primer and OSD design. The LAMP-OSD systems for MERS1B, BRAF and NRP2 were described in our previous study[22,23]. Design constraints used in engineering LAMP primers for human fecal *Bacteroides* 'HF183'[24,25] signature sequence are being published in a separate manuscript. Briefly, use of a forward PCR primer termed 'HF183' along with a relatively flexible set of reverse primers, such as BacR287, BFDRev, BthetR1[25,26], has been standardized for monitoring human fecal contamination of environmental water. We constrained the LAMP primer design software, Primer Explorer (Eiken, Tokyo, Japan), to use 'HF183' primer sequence as the F2 region of FIP. Additional specificity was ensured by limiting the B2 region of BIP to the target region that is typically recognized by the TaqMan probe used in the standard HF183/BthetR1 qPCR)[26]. RT-LAMP primers including FIP, BIP, F3, B3 and a loop primer for *Fusobacterium nucleatum* FN1868 RNA were also designed using the Primer Explorer software.

OSD probes for *Bacteroides* HF183 and *Fusobacterium nucleatum* FN1868 were designed using our previously published design rules[18]. Briefly, fluorescein-labeled OSD strands were designed to bind between F2 and F1 sequences or between the B2 and B1 sequences. The Iowa Black quencher-labeled OSD strand was designed to be partially complementary to the fluorophore-labeled strand. The lengths of the two strands were designed to ensure that the duplex region displayed a ΔG close to -18 kcal/mole (calculated using the NUPACK software suite) at 60 °C to 65 °C with salt concentration mimicking that of the LAMP reaction mix. Single-stranded toeholds at the 3'-end of fluorophore-labeled strands were designed to be 10 or 12 nucleotides long. All 3'-end -OH groups were blocked with inverted dT to prevent extension by DNA polymerase.

***In vitro* transcription.** Transcription templates were prepared by PCR amplification using Phusion DNA polymerase and plasmids bearing the transcription templates downstream of T7

RNA polymerase promoters. The PCR products were purified using Wizard SV PCR purification kit (Promega, Madison, WI, USA). Some 1000 ng of these double-stranded DNA transcription templates were transcribed using the HiScribe™ T7 High Yield RNA Synthesis Kit (NEB) according to the manufacturer's protocol. Transcription was allowed to occur at 37 °C for 2 h. Subsequently the transcription reactions were incubated with 4 units of DNase I (NEB) at 37 °C for 30 min to degrade the template DNA prior to RNA purification.

Denaturing polyacrylamide gel electrophoresis and RNA gel purification. Denaturing 8 % polyacrylamide gels containing 7 M urea were prepared using 40 % acrylamide and bis-acrylamide solution, 19:1 (Bio-Rad, Hercules, CA, USA) in 1X TBE buffer (89 mM Tris Base, 89 mM Boric acid, 2 mM EDTA, pH 8.0) containing 0.04% ammonium persulphate and 0.1% TEMED. An equal volume of 2X denaturing dye (7 M urea, 1X TBE, 0.1% bromophenol blue) was added to the RNA samples. These were incubated at 65 °C for 3 min followed by cooling to room temperature before electrophoresis. The gels were stained for 10 min with SYBR-Gold (Thermo Fisher) prior to visualization on the Storm Imager (GE Healthcare, Fairfield, CT, USA). For RNA purification, desired bands were excised from the gel and the RNA was eluted twice into TE (10:1, pH 7.5) buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8.0) by incubation at 70 °C and 1000 rpm for 20 min. Acrylamide traces were removed by filtering eluates through Ultrafree-MC centrifugal filter units (EMD Millipore, Billerica, MA, USA) followed by precipitation with 2X volume of 100% ethanol in the presence of both 15 µg glycogen and 0.3 M sodium acetate, pH 5.2. RNA pellets were washed once in 70% ethanol. Dried pellets of purified RNA were resuspended in 0.1 mM EDTA and stored at -80 °C.

LAMP-OSD and RT-LAMP-OSD with and without thresholding. Regular 25 µl LAMP-OSD reaction mixtures contained indicated amounts of true DNA templates along with a final concentration of 0.8 µM each of BIP and FIP primers, 0.2 µM each of B3 and F3 primers, and 0.4 µM of the loop primer. Amplification was performed in 1X Isothermal buffer (NEB) (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, pH 8.8) containing 1 M betaine, 0.4 mM dNTPs, 2 mM additional $MgSO_4$, 8 units of Bst 2.0 DNA polymerase, and 60 nM (or the indicated amount) of OSD reporter (unless otherwise indicated the OSD fluorophore-labeled strands were annealed with a 5-fold excess of the OSD quencher-labeled strands by incubation at 95 °C for 1 min followed by cooling at the rate of 0.1 °C/sec to 25 °C.). For real-time signal measurement 20 µl of these LAMP-OSD solutions were transferred into a 96-well PCR plate, which was maintained at 60 °C (HF183 and BRAF) or 65 °C (MERS1B, NRP2 and FN1868) for 1 h to 2.5 h. Fluorescence signals were recorded every 3 min with a LightCycler 96 real-time PCR machine (Roche, NC, U.S.A.). In assays designed to assess OSD saturation OSD reporter concentrations were increased to as high as 600 nM.

RT-LAMP-OSD assays for FN1868 RNA were performed using our previously described protocol[23]. Briefly, indicated amounts of RNA templates were amplified using 8 units of Bst 2.0 DNA polymerase and 2 units of AMV reverse transcriptase in the presence of 1.12 mM dNTPs, 1.6 µM each of FIP and BIP, 0.4 µM each of F3 and B3, 0.8 µM of the loop primer, 1X Thermopol buffer (NEB; 20 mM Tris-HCl, 10 mM $(NH4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, pH 8.8 at 25°C), 0.5X AMV RT buffer (NEB; 25 mM Tris-HCl, 37.5 mM potassium acetate, 4 mM magnesium acetate, 5 mM DTT, pH 8.3 at 25 °C), 60 nM of the OSD probe (60 nM fluorophore-labeled strand and 300 nM of the quencher-labeled strand), 2 mM additional $MgSO_4$, and 0.4 M Betaine. Fluorescence signals were recorded every 3 min with a LightCycler 96 real-time PCR machine (Roche, NC, U.S.A.).

For thresholded LAMP and RT-LAMP-OSD reactions the indicated copies of false target DNA or RNA were included along with the true targets in the reaction mixes described above. For all LAMP-OSD and RT-LAMP-OSD reactions with or without thresholding that were measured in real-time, templates were annealed with primers by incubation at 95 °C for 2 min followed by incubation on ice for 2 min prior to addition of OSD reporters and enzymes to initiate amplification. Performance of the HF183 and FN1868 LAMP-OSD and RT-LAMP-OSD assays with or without thresholding was also tested without subjecting the templates and primers to initial annealing as described above. Fluorescence output of these assays was measured both in real-time and at endpoint with smartphone imaging as described below. All experiments were performed at least in triplicate for statistical analysis.

Visual readout of LAMP-OSD and RT-LAMP-OSD fluorescence. For visual readout and smartphone imaging, LAMP-OSD and RT-LAMP-OSD reactions were modified to use more concentrated reagents in order to increase the fluorescence amplitude. A 25µL thresholded HF183 LAMP-OSD reaction mixture typically contained 1.6 µM each of BIP and FIP primers, 0.4 µM each of B3 and F3 primers, 0.8 µM of the loop primer, 1 M betaine, 0.8 mM dNTPs and 1X Isothermal Buffer (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, pH 8.8), additional 2 mM $MgSO_4$, 200 nM OSD reporter (200 nM fluorophore-labeled OSD strand and 240 nM quencher-labeled OSD strand) and 16 units of Bst 2.0 DNA polymerase. A 25 µl thresholded RT-LAMP OSD assay for FN1868 typically contained 120 nM OSD (120 nM fluorophore-labeled strands annealed with 600 nM quencher-labeled strands) and 8 units of Bst 2.0 DNA polymerase along with 2 units of AMV reverse transcriptase. For smartphone imaging the 25 µl reactions were assembled in transparent 0.2 ml individual PCR tubes and directly incubated for 60 min in a thermal cycler maintained at a constant temperature – 60 °C for HF183 and 65 °C for FN1868. The initial step of primer template annealing was not performed. The tubes were then placed on an UltraSlim-LED transilluminator (Syngene, Frederick, MD, USA) at room temperature to excite fluorescence and enable visual examination and imaging with an unmodified smartphone camera. In some cases duplicate reactions were also monitored in real-time using the LightCycler 96 real-time PCR machine.

Cultivation and RT-LAMP-OSD analysis of *Fusobacterium nucleatum*. *Fusobacterium nucleatum* ATCC 25586 was grown under anaerobic conditions in tryptic soy broth/agar + 0.5% yeast extract (TSBYE/TSAYE). Anaerobic conditions were maintained in a vinyl chamber (Coy, Grass Lake, Michigan, USA) with the following atmosphere: 85% nitrogen, 10% carbon dioxide, and 5% hydrogen. Liquid cultures were shaken at 250 rpm, and all media were pre-reduced overnight in the chamber (with shaking at 250 rpm for liquid media) prior to usage. An overnight stationary-phase culture of *F. nucleatum* was diluted to an A_{600} of 0.1. Then, lysine (or an equal volume of sterile water for the control) was added at a final concentration of 20 mM. After being further grown for 2 hours, the cultures were removed from the chamber and subjected to 10-fold serial dilution in water. Some 6 µl aliquots of chosen dilutions were directly added to FN1868 thresholded RT-LAMP OSD reactions that were then incubated at 65 °C for 1h. Smartphone images of accumulated fluorescence at reaction endpoint were acquired as described above. Colony forming units (CFU)/ml were quantified by plating serial dilutions on TSBYE medium in duplicate followed by incubation in the anaerobic chamber.

Thresholded LAMP-OSD analysis of sewage-contaminated water. Primary raw sewage was collected from the Walnut Creek Wastewater Treatment Plant (Austin, TX, USA). The sample was stored at 4 °C upon collection and analyzed within 6 h. Artificially-contaminated water samples

were prepared by spiking 50 µl of primary sewage to a volume of 200 µl sterile water followed by vortexing to ensure uniform mixing. Estimation of HF183 copies in these samples was performed by adding some 3 µl of this diluted sewage sample directly into thresholded HF183 LAMP-OSD reactions and incubating them at 60 °C for 1 h. Smartphone images of accumulated fluorescence were acquired as detailed above.

Real-time PCR analysis of HF183. The standard TaqMan qPCR protocol for HF183 analysis was used as previously described[26]. Briefly, each qPCR reaction contained 3.5 µl of Primer/Probe mix (0.093 µM TaqMan probe, 1.2 µM BthetR1 primer and 1.2 µM HF183-1 primer), 6 µl water and 12.5 µl 2×FastStart Essential DNA Probes Master (Roche, NC, U.S.A.). To this mixture 3 µl of water, or pure plasmid templates or sewage-contaminated water samples were added. The qPCR amplification and analysis was performed using the LightCycler 96 real-time PCR machine (Roche). The cycling conditions included 2 min of pre-heating at 50 °C followed by 10 min of pre-heating at 95 °C, and then 50 cycles of 15 sec at 95 °C followed by 1 min at 60 °C. All experiments were performed at least in triplicate for statistical analysis.

Supplementary Table S1. Primers and reporters used in this paper.

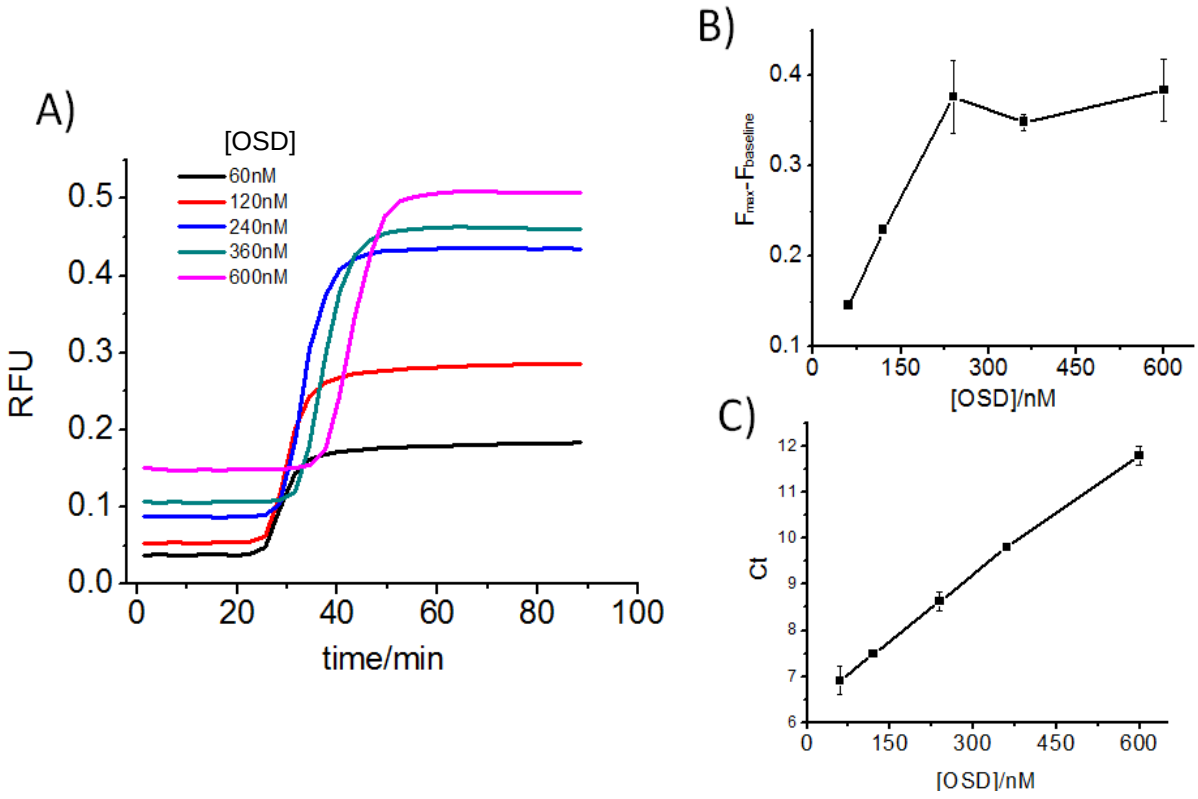
Name		Sequence (5'-3')
MERS1B primers	**F3**	ACAGTTCCTGGATATCCTAAG
	B3	CTCAGTGTCTACAACACCA
	FIP	AGCACCCTCAACATCGAAGCACTCGTGAAGAGGCTGTA
	BIP	TGCTTCCCGTAATGCATGTGGACTGGCTGAACAACAAAGT
	LP	CTATCCAGCTTCGAACTTGCCT
MERS1B OSD reporter	**Reporter F**	/56FAM/CACACCAGTTGAAAATCCTAATTGTAGAGGCACATTGGTG/3InvdT/
	Reporter Q	CTCTACAATTAGGATTTTCAACTGGTGTG/3IABkFQ/
BRAF primers	**FIP**	ACTGATGGGACCCACTCCATAAGACCTCACAGTAAAAATA
	BIP	AACAGTTGTCTGGATCCATTTTGTGACATCTGACTGAAAGCTGTA
	F3	CCACAGAGACCTCAAGAGT
	B3	ACAGAACAATTCCAAATGCATAT
BRAF OSD reporter	**Reporter F**	GATTTCACTGTAGCTAGACCAAAATCACCTATCGA/36-FAM/
	Reporter Q	/5IABkFQ/TCGATAGGTGATTTTGGTCTAGCT/3InvdT/
***NRP2* primer**	**FIP**	GCTTCTACTGTGGGCCGAACTCGAGCTGTCAAGCCCAAGG
	BIP	CCTCTTCACCCTCCCATGATGCTCTGCAGGCTACTTCTGGAA
	F3	CTCATTGGCACAGTGGTAGT
	B3	CCTGGAGTGGCTTTCAAACT
***NRP2* OSD reporter**	**Reporter F**	/56-FAM/ATCCCTAGGACTTACCAGCTTTCTGAACACTGC/3InvdT/
	Reporter Q	AAAGCTGGTAAGTCCTAGGGAT/3IABkFQ/
HF183 primer[a]	**FIP**	GAACGCATCCCCATCGTCTACCATCATGAGTTCACATGTCCG
	BIP	GGTAACGGCCCACCTAGTCAACCCGTGTCTCAGTTCCAATGT
	F3	GCCAGCCTTCTGAAAGGAAG
	B3	TGCCTCCCGTAGGAGTTTG
	LP	GGAAAATACCTTTAATCATG
HF183 OSD reporter[a]	**Reporter F**	GATGGATAGGGGTTCTGAGAGGAAGGTCCC AGC/36-FAM/
	Reporter Q	/5IABkFQ/GCTGGGACCTTCCTCTCAGAAC/3InvdT/
FN1868 primers	**FIP**	CAAAACCAACTCTTACATGTCCTCCCAGGAGTTGGAAGACATCA
	BIP	ACATAGACAAGGGAATCTTAGCAAATTCTCTTCCTAGCTCCTTTG
	F3	AGGTTCAACTTGGACAGTT
	B3	TTGTCTTGCTTCATCAGGA
	LP	TCAAATGGAGAATTAGTTGAAAGAGTTGTAAG
FN1868 OSD reporter	**Reporter F**	/56-FAM/CAGATTCCAAATGGCGGCCTTAGCAATAGTTATG/3InvdT/
	Reporter Q	TAAGGCCGCCATTTGGAATCTG/3IABkFQ/

[a] Manuscript describing the development and validation of the HF183 LAMP-OSD assay is in preparation.

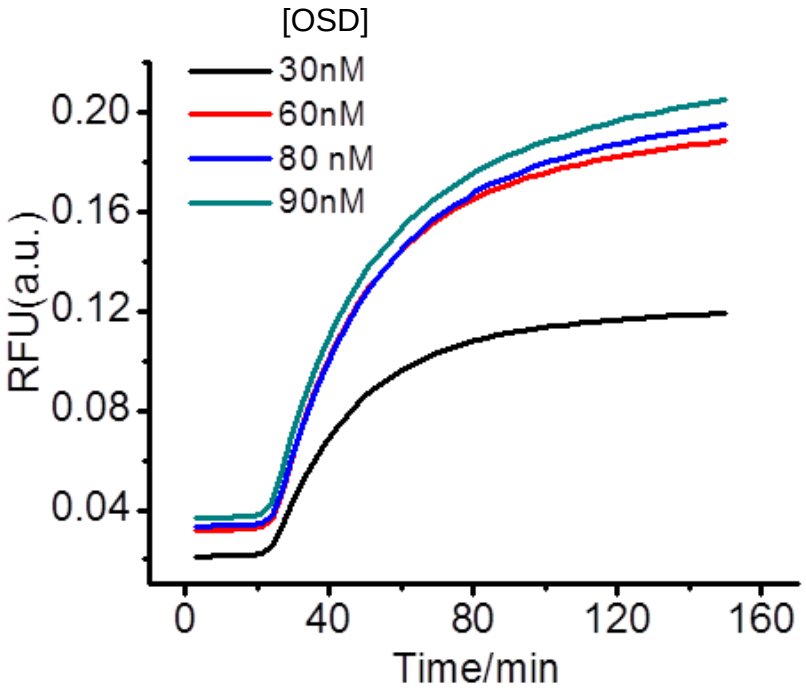
Supplementary Table S2. Target and false target sequences.

True (T_T) or False (T_F) Target	Sequence[a]
FN1868 T_T	TAATACGACTCACTATAGGGATATCAAAAACAAGGATTTATACAAAAACCTATGCATTTTGATT TTGTATTGGGTGTACAAATGTCTGCTTCTGCAAGAGATTTAGTATTTATGTCAGAAAGTATTCCA GAAGGTTCAACTTGGACAGTTGCAGGAGTTGGAAGACATCAATTCCAAATGGCGGCCTTAGCA ATAGTTATGGGAGGACATGTAAGAGTTGGTTTTGAAGATAATGTATACATAGACAAGGGAATCT TAGCAAAATCAAATGGAGAATTAGTTGAAAGAGTTGTAAGATTAGCAAAGGAGCTAGGAAGAG AAATTGCAACTCCTGATGAAGCAAGACAAATATTAAGTTT
FN1868 T_F	TAATACGACTCACTATAGGGATATCAAAAACAAGGATTTATACAAAAACCTATGCATTTTGATT TTGTATTGGGTGTACAAATGTCTGCTTCTGCAAGAGATTTAGTATTTATGTCAGAAAGTATTCCA GAAGGTTCAACTTGGACAGTTGCAGGAGTTGGAAGACATCAGGTTGGTCGACAATTAACGAAC TGTACTTCAGGAGGACATGTAAGAGTTGGTTTTGAAGATAATGTATACATAGACAAGGGAATCT TAGCAAAATCAAATGGAGAATTAGTTGAAAGAGTTGTAAGATTAGCAAAGGAGCTAGGAAGAG AAATTGCAACTCCTGATGAAGCAAGACAAATATTAAGTTT
HF183 T_T	TTGGCCAGCCTTCTGAAAGGAAGATTAATCCAGGATGGGATCATGAGTTCACATGTCCGCATGA TTAAAGGTATTTTCCGGTAGACGATGGGGATGCGTTCCATTAGATAGTAGGCGGGGTAACGGCC CACCTAGTCAACGATGGATAGGGGTTCTGAGAGGAAGGTCCCCCACATTGGAACTGAGACACG GTCCAAACTCCTACGGGAGGCAGCAGTGAGGAATATTGGTCAATGGGCGATGGCCTGAACCAG CCAAGTAGCGTGAAGGATGACTGCCCTATGG
HF183 T_F	TTGGCCAGCCTTCTGAAAGGAAGATTAATCCAGGATGGGATCATGAGTTCACATGTCCGCATGA TTAAAGGTATTTTCCGGTAGACGATGGGGATGCGTTCCATTAGATAGTAGGCGGGGTAACGGCC CACCTAGTCAACGGCGCCATGTGGAGCATGTGGTAAGCGTAACCACATTGGAACTGAGACACG GTCCAAACTCCTACGGGAGGCAGCAGTGAGGAATATTGGTCAATGGGCGATGGCCTGAACCAG CCAAGTAGCGTGAAGGATGACTGCCCTATGG
MERS1B T_T	TAATACGACTCACTATAGGGCGTGAATCTTAATTTACCCGCAAATGTCCCATACTCTCGTGTTAT TTCCAGGATGGGCTTTAAACTCGATGCAACAGTTCCTGGATATCCTAAGCTTTTCATTACTCGTG AAGAGGCTGTAAGGCAAGTTCGAAGCTGGATAGGCTTCGATGTTGAGGGTGCTCATGCTTCCCG TAATGCATGTGGCACCAATGTGCCTCTACAATTAGGATTTTCAACTGGTGTGAACTTTGTTGTTC AGCCAGTTGGTGTTGTAGACACTGAGTCCT GAC GAG TGG GTT TAA CG
MERS1B T_F	TAATACGACTCACTATAGGGCGTGAATCTTAATTTACCCGCAAATGTCCCATACTCTCGTGTTAT TTCCAGGATGGGCTTTAAACTCGATGCAACAGTTCCTGGATATCCTAAGCTTTTCATTACTCGTG AAGAGGCTGTAAGGCAAGTTCGAAGCTGGATAGGCTTCGATGTTGAGGGTGCTCATGCTTCCCG TAATGCATGTGGAGCTTTAGGATATCCATTAATTTGATCACCCGGACGCGTTAACTTTGTTGTTC AGCCAGTTGGTGTTGTAGACACTGAGTCCT GAC GAG TGG GTT TAA CG
NRP2 T_T	CACTCATTGGCACAGTGGTAGTTAGAGGTGAAAAGTAGAGCTGTCAAGCCCAAGGGCTTAGCTT TAGGGCTCCTCCTGAGTTCGGCCCACAGTAGAAGCAAGATTTTAACTAGCCCCTTTTCCTCTTCA CCCTCCCATGATGCGCAGTGTTCAGAAAGCTGGTAAGTCCTAGGGATTTCCAGAAGTAGCCTGC AGAAGAAGGTAAGTTTGAAAGCCACTCCAGGGGTCCTGATGCTGTCATGCTCAGTGAGCCATTT TACAGTTCTCCAAAGTCTAGCCCTGTTTCGGACCTGCACTTCACCTCTAAGTTATGTACAACTCA ACC
NRP2 T_F	CACTCATTGGCACAGTGGTAGTTAGAGGTGAAAAGTAGAGCTGTCAAGCCCAAGGGCTTAGCTT TAGGGCTCCTCCTGAGTTCGGCCCACAGTAGAAGCAAGATTTTAACTAGCCCCTTTTCCTCTTCA CCCTCCCATGATGCATAAGAGAGATTGGGCCTCTTGAATGAGGTCCGTTCCAGAAGTAGCCTGC AGAAGAAGGTAAGTTTGAAAGCCACTCCAGGGGTCCTGATGCTGTCATGCTCAGTGAGCCATTT TACAGTTCTCCAAAGTCTAGCCCTGTTTCGGACCTGCACTTCACCTCTAAGTTATGTACAACTCA ACC
BRAF T_T	CCACAGAGACCTCAAGAGTAATAATATATTTCTTCATGAAGACCTCACAGTAAAAATAGGTGAT TTTGGTCTAGCTACAGTGAAATCTCGATGGAGTGGGTCCCATCAGTTTGAACAGTTGTCTGGAT CCATTTTGTGGATGGCACCAGAAGTCATCAGAATGCAAGATAAAAATCCATACAGCTTTCAGTC AGATGTATATGCATTTGGAATTGTTCTGT
BRAF T_F	CCACAGAGACCTCAAGAGTAATAATATATTTCTTCATGAAGACCTCACAGTAAAAATACCAACA TGACCCAGACCGGCACCACCGAATCGATGGAGTGGGTCCCATCAGTTTGAACAGTTGTCTGGAT CCATTTTGTGGATGGCACCAGAAGTCATCAGAATGCAAGATAAAAATCCATACAGCTTTCAGTC AGATGTATATGCATTTGGAATTGTTCTGT

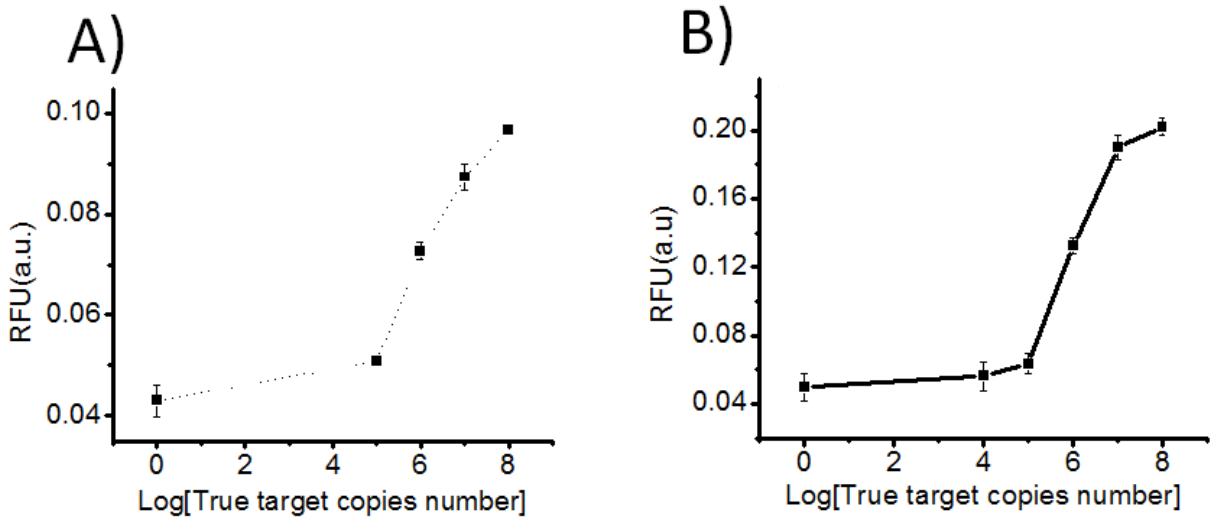
[a] Sequences in green represent OSD-binding sites in true targets. Sequences shown in red represent the corresponding randomized region in false targets that lack complementarity to OSD reporters.



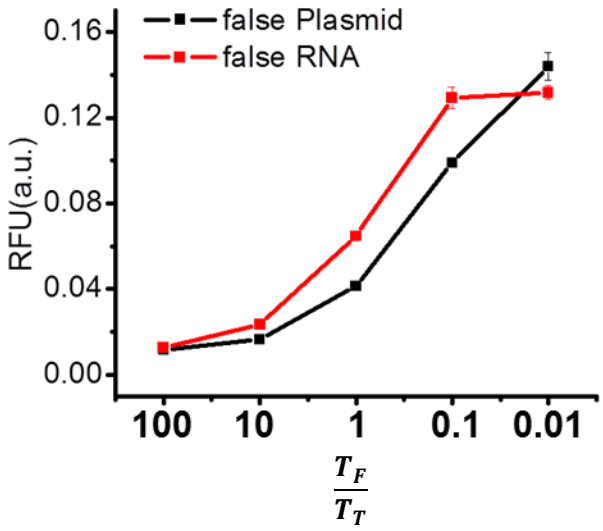
Supplementary Figure S1. Effect of OSD concentration on the HF183 LAMP reaction. A) Real-time fluorescence accumulation in HF183 LAMP reactions with different amounts of OSD reporters. All other reaction conditions remained constant. B) Effect of HF183 OSD concentration on endpoint fluorescence of LAMP. Background-subtracted endpoint fluorescence values from plot A are depicted. C) Influence of OSD concentration on the time-to-signal (Ct) of HF183 LAMP-OSD reaction.



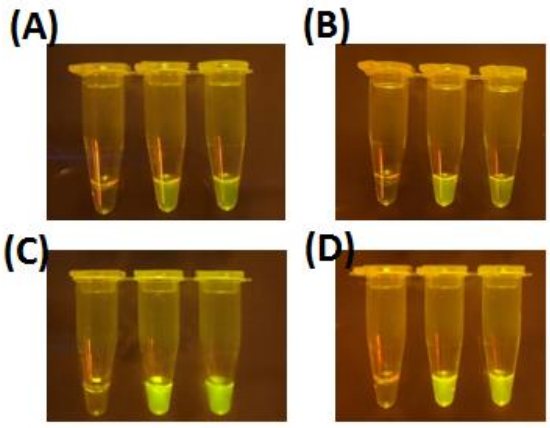
Supplementary Figure S2. Real-time MERS1B LAMP-OSD assays containing the indicated amounts of OSD reporters. All other reaction conditions were kept the same. Fluorescence accumulation over time is depicted.



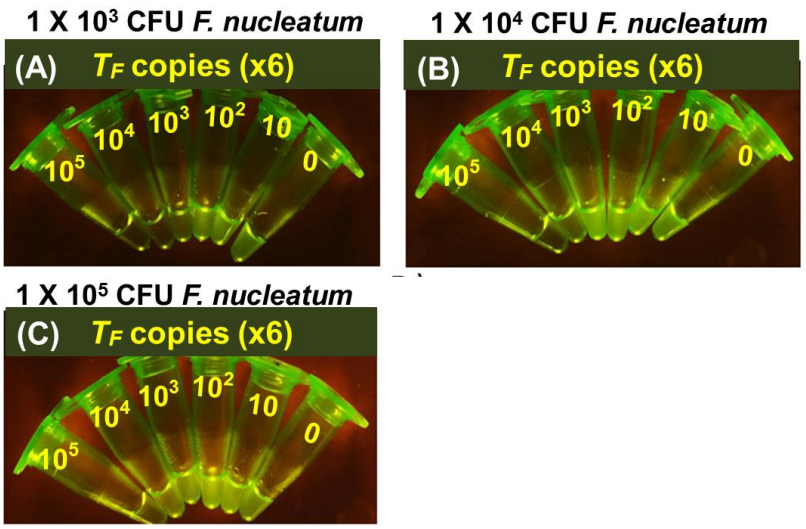
Supplementary Figure S3. Thresholded LAMP-OSD assays for A) BRAF and B) NRP2 pure DNA templates. In both cases 0 to 10^8 copies of true targets were amplified at 60 °C in the presence of 10^6 false targets. End-point fluorescence measured at 37 °C using the LightCycler 96 real-time PCR machine is depicted on the Y-axes.



Supplementary Figure S4. Thresholded FN1868 RT-LAMP-OSD assays with false RNA targets (red trace) or false plasmid DNA target (black trace). Endpoint fluorescence is depicted on the Y-axis.



Supplementary Figure S5. Smartphone images of HF183 LAMP-OSD assays performed with different amounts of HF183 plasmid templates and OSD reporters. In each panel, tubes from left to right contain 0, 2000 and 20 copies of HF183. Panels A through D contain 60 nM, 120 nM, 200 nM and 240 nM, respectively of OSD fluorophore-labeled strands annealed with 1.2-fold excess of quencher-labeled strands. LAMP-OSD reactions were performed for 1h at 60 °C and images were captured immediately afterwards using a Transilluminator at room temperature.



Supplementary Figure S6. Thresholded RT-LAMP-OSD analysis of FN1868 RNA in 10^3 CFU (A), 10^4 CFU (B) and 10^5 CFU (C) of log phase *Fusobacterium nucleatum* bacteria. The number of false FN1868 plasmid copies (*from left to right*: $6x10^5$, $6x10^4$, $6x10^3$, $6x10^2$, 60 and 0) used to threshold the assays in panels A through C are depicted in yellow atop each tube.