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SUPPLEMENTARY INFORMATION

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A CRISPR-based assay for the detection of opportunistic infections post-transplantation and for the monitoring of transplant rejection

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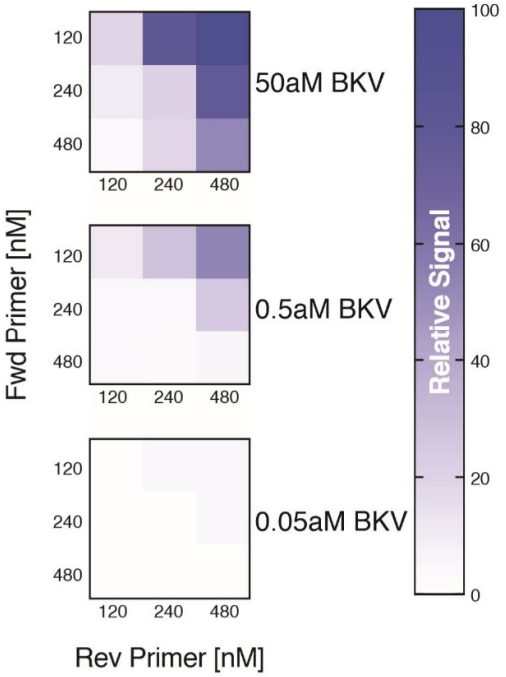
Contents:

Figure S1

a

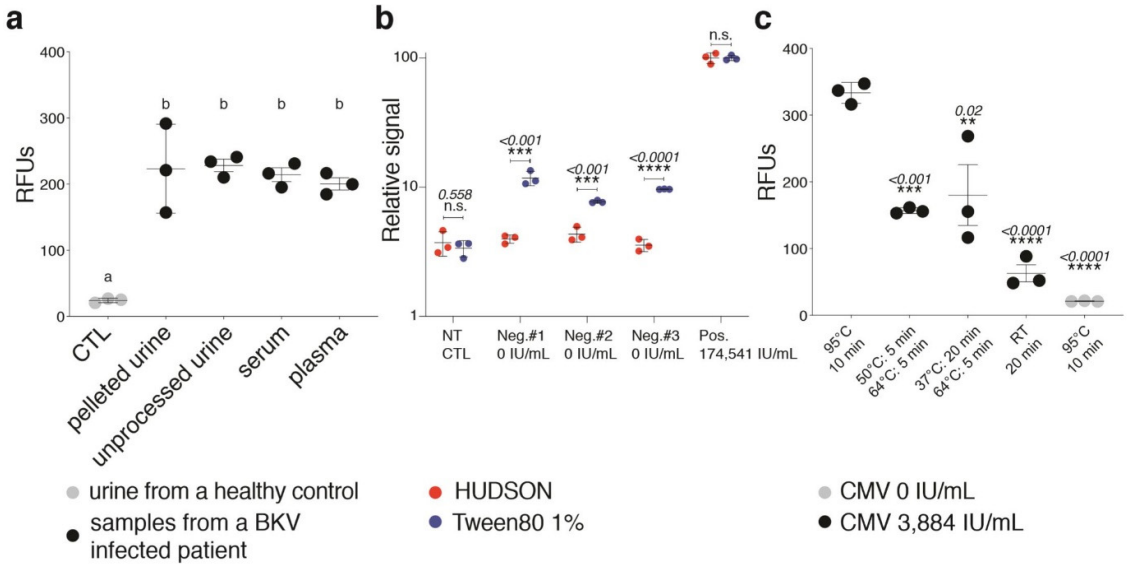
BKV	CMV	Rejection marker
STA	UL54	CXCL9
VP2	UL55	CXCL10
VP3	UL83	
	UL97	

b



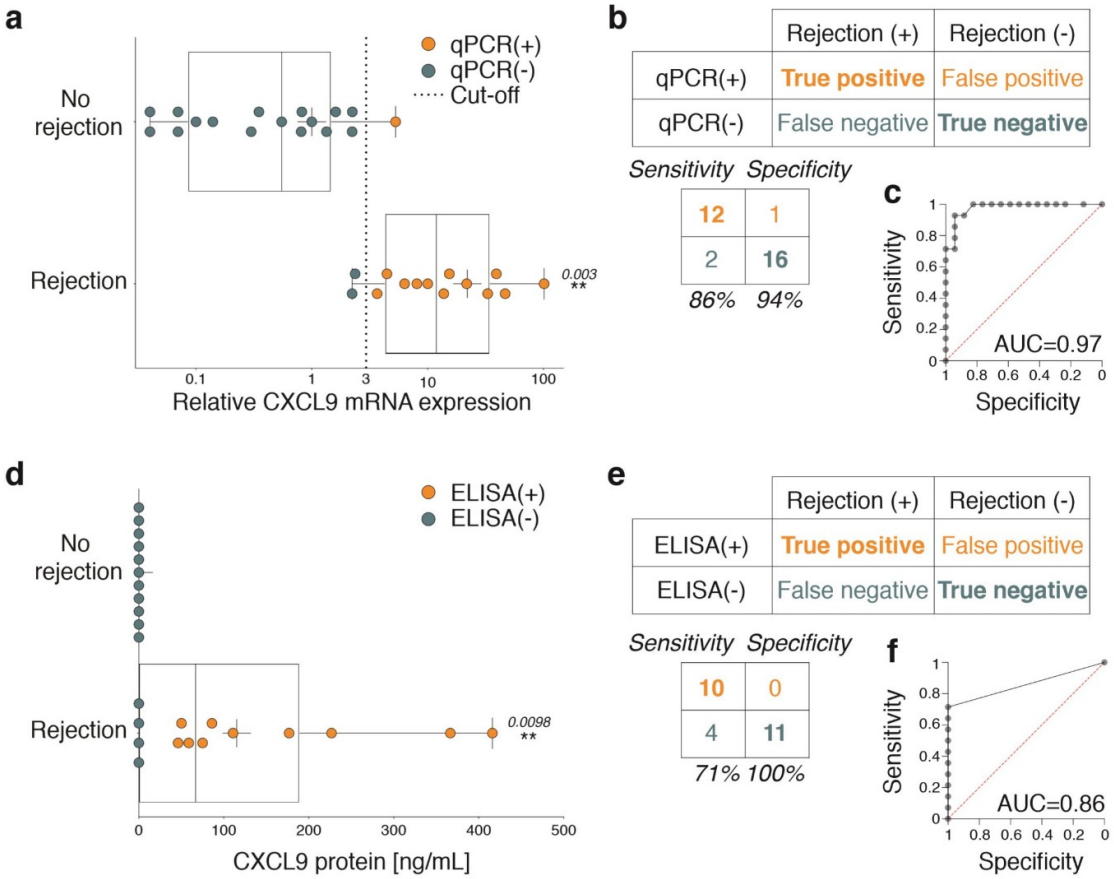
Supplementary Figure 1 Target genes and primer optimization. a, Target genes tested for detection of BKV or CMV infection and rejection. **b**, RPA primer dilution matrix. The Sherlock fluorescence signal of BKV detection (ATCC synthetic standard) at the indicated target concentrations is depicted as colour intensity relative to the highest signal (100). Forward and reverse primer concentrations for RPA as indicated.

Figure S2



Supplementary Figure 2 Comparison of different specimen types and sample processing. a, Detection of BKV in the indicated specimens from the same patient compared to urine from a healthy control. Different small letters indicate significant differences as assessed by one-way ANOVA and Tukey's multiple comparisons test. Symbols: mean ± SD of 3 independent reactions. **b**, Comparison of sample processing by the HUDSON method (red circles) and incubation with Tween80 1% for 20 min at room temperature (blue circles). Tested on 3 different CMV negative patient samples to test for unspecific background noise and one CMV positive patient sample. Fluorescence was normalized to the highest signal (HUDSON, 100). Asterisks indicate significant differences as assessed by Student's two-tailed t-test. Symbols: mean ± SD of 3 independent reactions. **c**, Comparison of different HUDSON-based protocols on a CMV negative (grey circles) and a CMV positive (black circles) patient sample. Asterisks indicate significant differences to the 95°C/10 min (3884 IU/mL) condition as assessed by one-way ANOVA and Dunnett's multiple comparisons test. Symbols: mean ± SD of 3 independent reactions. n.s. not significant, $p<0.05$ (*), $p<0.01$ (**), $p<0.001$(***), $p<0.0001$ (****) (**b**,**c**).

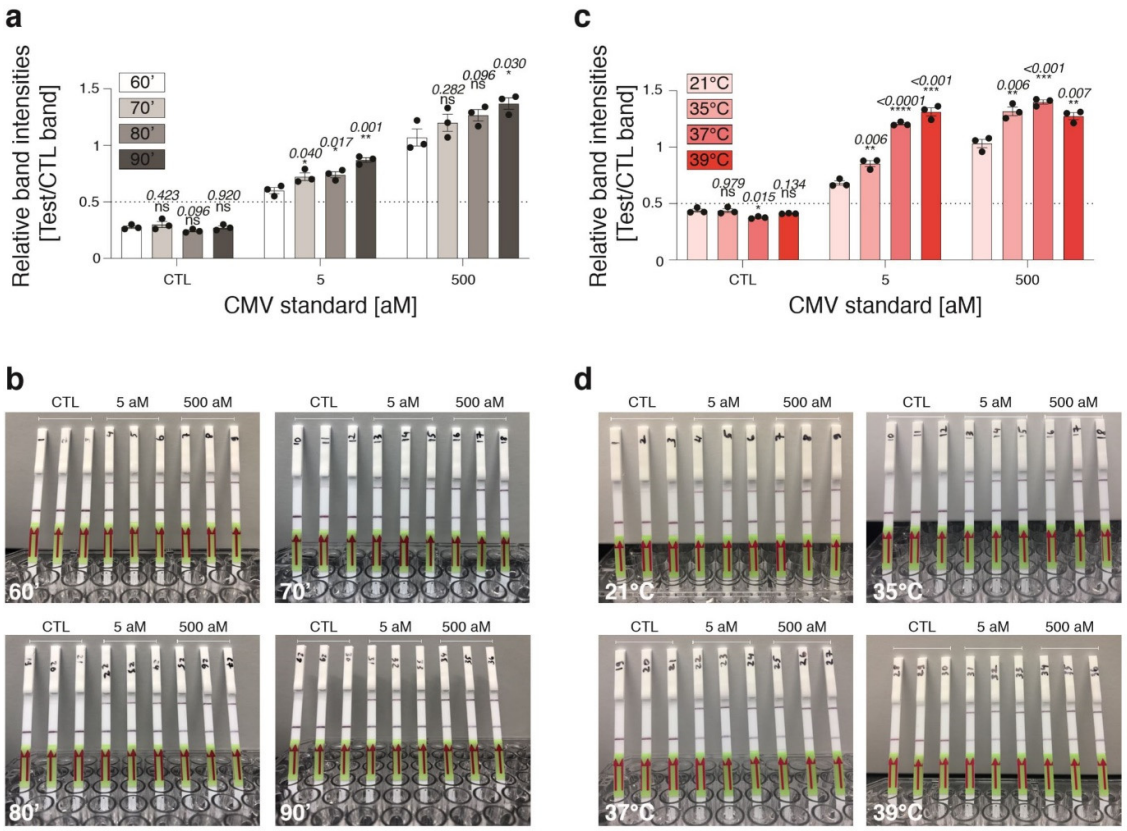
Figure S3



Supplementary Figure 3 CXCL9 mRNA and protein levels in rejection patients and controls. a, qPCR-based detection of CXCL9 mRNA in rejection patients (n=14) and no rejection control patients (n=17). Blue circles indicate qPCR negative tests and orange circles indicate qPCR positive test results. The dashed line indicates the cut-off differentiating between a negative and positive test result. **b**, Sensitivity and specificity of rejection detection by qPCR calculated using the cut-off value depicted in (a). **c**, Area under the receiver-operating-characteristic (ROC) curve (AUC) assessing the accuracy of qPCR-based rejection diagnostics (1 indicates perfect discriminatory value; 0.5 or less indicates no discriminatory value). **d**, ELISA-based detection of CXCL9 protein in rejection patients (n=14) and no rejection control patients (n=11). The tested samples were the same as depicted in (a) (rejection) or a subset of them (no rejection). Blue circles indicate ELISA negative tests and orange circles indicate ELISA positive test results. **e**, Confusion matrix indicating the sensitivity and specificity of ELISA-based rejection detection. **f**, Area under the receiver-operating-characteristic (ROC) curve (AUC) assessing the accuracy of ELISA-based rejection diagnostics (1 indicates perfect discriminatory value; 0.5 or less indicates no discriminatory value).

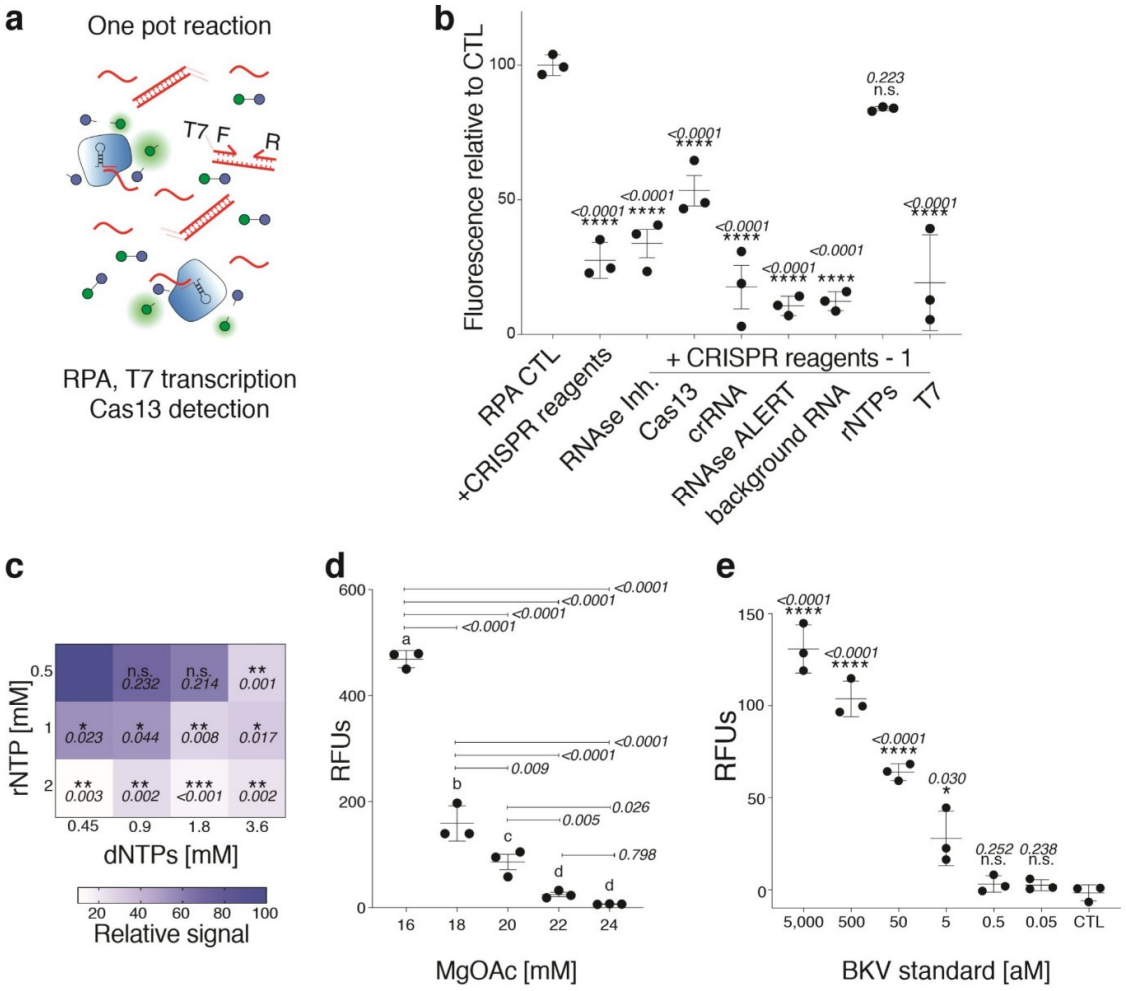
Box plot lines: median and quartiles, whiskers: data range, crosses: averages. Each symbol represents a different, independent patient sample. Asterisks: significant difference to control as assessed by Student's two-tailed t-test. $p<0.01$ (**) (**a**,**d**)

Figure S4



Supplementary Figure 4 Influence of temperature and incubation time on lateral-flow band intensity. a, Incubation of the CRISPR reaction detecting the CMV synthetic standard at the indicated concentrations for the indicated reaction times. The lateral-flow-based readout was quantified as the ratio of test/control band. The dashed line indicates the assay's cut-off. Symbols: mean ± SD of 3 independent reactions. Asterisks indicate significant differences to the 60 min control reaction time as assessed by Student's two-tailed t-test. **b**, Images of the lateral-flow assays quantified in (a). **c**, Incubation at different temperatures for the detection of the CMV synthetic standard at the indicated concentrations with lateral flow. Symbols: mean ± SD of 3 independent reactions. Asterisks indicate significant differences to the 21°C control reaction as assessed by Student's two-tailed t-test. **d**, Images of the lateral-flow assays quantified in (c). n.s. not significant, $p<0.05$(*), $p<0.01$ (**), $p<0.001$(***), $p<0.0001$ (****).

Figure S5



Supplementary Figure 5 Optimization of the one-pot reaction. a, Schematic illustration of the one-pot assay. **b**, Effect of CRISPR and T7 transcription reagents upon addition to the RPA reaction. CRISPR reagents were added pooled (+CRISPR reagents) or as a pool with one component missing (+CRISPR reagents -1). CMV synthetic DNA served as target (500aM). A separate CRISPR/T7 reaction served as readout. Fluorescence was normalized to the highest signal (RPA CTL, 100). Asterisks indicate significant differences to the RPA control without any additions (RPA CTL) as assessed by one-way ANOVA and Dunnett's multiple comparisons test. Symbols: mean ± SD of 3 independent reactions. **c**, Concentration matrix of rNTPs and dNTPs in the one-pot reaction. The fluorescence signal is depicted as colour intensity relative to the highest signal. Asterisks indicate significant differences as assessed by Student's two-tailed t-test. **d**, Testing of the indicated MgOAc concentrations in the one-pot reaction. CMV synthetic DNA served as target (500aM). Different letters indicate significant differences between groups as assessed by one-way ANOVA and Tukey's multiple comparisons test. Symbols: mean ± SD of 3 independent reactions. **e**, One-pot reaction detecting the ATCC quantitative BKV synthetic standard (Dunlop strain) at the indicated concentrations. Symbols: mean ± SD of 3 independent reactions. Asterisks indicate significant differences to no template control (CTL) as assessed by Student's two-tailed t-test. p<0.05 (*), p<0.01 (**), p<0.001(***), p<0.0001 (****).

Table S1

Characteristics	All subjects (N=31)	Rejection (n=14)	No Rejection (n=17)	p-value
Recipient Age (years)				
Mean ± SD	59 ± 13	61 ± 12	57 ± 14	0.589
Recipient Gender				
Female (n, %)	17 (54.8%)	5 (35.7%)	12 (70.6%)	0.076
Male (n, %)	14 (45.2%)	9 (64.3%)	5 (29.4%)	
Recipient Race				
African American (n, %)	10 (32.2%)	7 (50.0%)	3 (17.6%)	0.131
Caucasian (n, %)	14 (45.1%)	4 (28.6%)	10 (58.8%)	
Other / Unknown	7 (22.6%)	3 (21.4%)	4 (23.6%)	
Donor gender				
Female (n, %)	20 (64.5%)	9 (64.3%)	11 (64.7%)	0.999
Male (n, %)	11 (35.5%)	5 (35.7%)	6 (83.3%)	
Donor Source				
Living (n, %)	11 (35.5%)	4 (28.6%)	7 (41.2%)	0.707
Deceased (n, %)	20 (64.5%)	10 (71.4%)	10 (58.8%)	
Number of HLA mismatches				
Mean ± SD	4.4 ± 1.2	4.2 ± 0.9	4.6 ± 1.4	0.096
Induction Therapy				
Thymoglobulin (n, %)	16 (51.6%)	5 (35.7%)	11 (64.7%)	0.268
Basiliximab (n, %)	14 (45.2%)	8 (57.1%)	6 (35.3%)	
Alemtuzumab (n, %)	1 (3.2%)	1 (7.2%)	0 (0.0%)	
Time since transplant (months)				
Mean ± SD	12 ± 12	14 ± 13	11 ± 12	0.256
Cause of kidney disease				
Diabetes	7 (22.6%)	4 (28.5%)	3 (17.6%)	0.464
Polycystic kidney disease	5 (16.2%)	3 (21.5%)	2 (11.8%)	
Glomerulopathy	8 (25.8%)	3 (21.5%)	5 (29.5%)	
Interstitial Nephritis	3 (9.6%)	0 (0.0%)	3 (17.6%)	
Other/Unknown	8 (25.8%)	4 (28.5%)	4 (23.5%)	

Supplementary Table 1. Baseline and demographic characteristics of kidney transplanted patients. Baseline characteristics are presented as mean ± SD. For non-categorical variables, data were analysed using Mann-Whitney test. For categorical variables, data were analysed using Fisher's exact test.

Table S2

Characteristics	n=14
Creatinine (mg/dL)	3.24 ± 1.63
eGFR (ml/min/1.73m^2)	23.21 ± 9.27
Rejection Type (n, %)	
Borderline	1 (7.2%)
IA/IB	8 (57.1%)
IIA/IIB	4 (28.5%)
III	1 (7.2%)
Banff g score	1.5 ± 1.4
Banff i score	2.4 ± 0.8
Banff t score	2.1 ± 0.8
Banff v score	0.6 ± 0.9
Banff ptc score	1.5 ± 1.3
Banff ci score	1.2 ± 0.7
Banff ct score	1.2 ± 1.0
Banff cv score	1.2 ± 1.0
Banff cg score	0.6 ± 0.9
Banff ah score	0.6 ± 0.9
C4d score	0.6 ± 1.2

Supplementary Table 2. Diagnosis at the time of biopsy from rejection patients' cohort. Data is expressed as mean ± SD. Banff score abbreviations: glomerulitis (g), interstitial inflammation (i), tubulitis (t), intimal arteritis (v) peritubular capillaritis (ptc), interstitial fibrosis (ci), tubular atrophy (ct), vascular fibrous intimal thickening (cv), glomerular basement membrane double contours (cg), arteriolar hyalinosis (ah).

Table S3

	ELISA	Biolayer interferometry	CRISPR diagnostics	qPCR
Reference	RND systems (DCX900) Hricik et al., 2013	Gandolfini et al., 2017	this paper	Altona diagnostics, RealStar Kits 021003 (CMV); 031003 (BKV)
Analyte	CXCL9 protein	CXCL9 protein	CXCL9 mRNA; BKV & CMV DNA	BKV & CMV DNA
Speed				
Assay length	4h 30min	60min	108min	85min
Hands-on time	1h 20min	10min	10min	10min
Costs				
Equipment	> $5,000 USD	> $100,000 USD (OctetRED96)	> $5,000 USD (fluorescence) $2.3 USD per test (lateral flow)	> $5,000 USD
Reagents (per test)	$5.4 USD	$1 USD	$1 USD	$20 USD
Sensitivity	11.3 pg/mL	35 pg/mL	low attomolar range	low attomolar range
POCT compatibility				
Isothermal incubation	Yes	Yes	Yes	No
Minimal equipment	No	No	Yes (lateral flow)	No
Visual output	No	No	Yes (lateral flow)	No

Supplementary Table 3. Comparison of CRISPR diagnostics with ELISA, biolayer interferometry and qPCR for the detection of CXCL9, BKV and CMV.

Table S4

Name	Sequence (5' – 3')
RPA Primer	
BKV_STA_fwd	GAAATTAATACGACTCACTATAGGCATTGCAGAGTTTC TTCAGTTAGGTCTAAGCC
BKV_STA_rev	AATTTTTAAGAAAAGAGCCCTTGGTTTGGATA
CMV_UL54_fwd	GAAATTAATACGACTCACTATAGGGCACCAGCCGAAC GTGGTGATCCGCCGATCGATGAC
CMV_UL54_rev	CTATCAGCAACTGGACCATGGCCAGAAAAATCG
CXCL9_fwd	GAAATTAATACGACTCACTATAGGTATCCACCTACAAT CCTTGAAAGACCTTAAAC
CXCL9_rev	TTAGACATGTTTGAACTCCATTCTTCAGTGTA
qPCR Primer and Probes	
CXCL9_fwd	CTTTTCCTCTTGGGCATCATCT
CXCL9_rev	AGGAACAGCGACCCTTTCTCA
CXCL9 probe	FAM-TACTGGGGTTCCTTGCACTCCAATCAGA-TAMRA
18S_fwd	GCCCGAAGCGTTTACTTTGA
18S_rev	TCCATTATTCCTAGCTGCGGTATC
18S_probe	FAM-AAAGCAGGCCCGAGCCGCC-TAMRA
Oligos for T7 synthesis of crRNAs	
T7_fwd	GAAATTAATACGACTCACTATAGG
BKV_STA_rev	CTGTGTGAAGCAGTCAATGCAGTAGCAAGTTTTAGTCC CCTTCGTTTTTGGGGTAGTCTAAATCCCTATAGTGAGT CGTATTAATTTC
CMV_UL54_rev	CGCGTCAGCGGATCCACACGGACCTCGTGTTTTAGTC CCCTTCGTTTTTGGGGTAGTCTAAATCCCTATAGTGAG TCGTATTAATTTC
CXCL9_rev	GCCCTTCCTGCGAGAAAATTGAAATCATGTTTTAGTCC CCTTCGTTTTTGGGGTAGTCTAAATCCCTATAGTGAGT CGTATTAATTTC
Synthetic targets	
CXCL9	GAAATTAATACGACTCACTATAGGATGAAGAAAAGTGG TGTTCTTTTCCTCTTGGGCATCATCTTGCTGGTTCTGAT TGGAGTGCAAGGAACCCCAGTAGTGAGAAAGGGTCG CTGTTCCTGCATCAGCACCAACCAAGGGACTATCCAC CTACAATCCTTGAAAGACCTTAAACAATTTGCCCCAAG CCCTTCCTGCGAGAAAATTGAAATCATTGCTACACTGA AGAATGGAGTTCAAACATGTCTAAACCCAGATTCAGCA GATGTGAAGGAACTGATTAAAAAGTGGGAGAAACAGG TCAGCCAAAAGAAAAAGCAAAAGAATGGGAAAAAACAT CAAAAAAAGAAAGTTCTGAAAGTTCGAAAATCTCAACG TTCTCGTCAAAAGAAGACTACATAA
Cleavage Reporter	
Lateral Flow	6FAM-mArArUrGrGrCmAmArArUrGrGrCmA-BIO
Fluorescence	RNAse ALERT V2 (Thermo)
	Red indicates the T7 promoter sequence