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Correction: Review: a comprehensive summary of a decade development of the recombinase polymerase amplification

Jia Li,^a Joanne Macdonald ^{*b,c} and Felix von Stetten ^{*a,d}

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Correction for 'Review: a comprehensive summary of a decade development of the recombinase polymerase amplification' by Jia Li *et al.*, *Analyst*, 2019, **144**, 31–67.

The authors regret that the original version of the review contained some incorrect data. Corrections to the original article are listed as follows.

Some of the values in Table 5 were incorrect. The corrected version of Table 5 is presented below.

In section 2.3, data from ref. 41 were not cited correctly and should be removed. The respective passage should read as follows: "However, shorter amplicons (79 nucleotides;³⁷ 94 nucleotides^{38–40}) and longer amplicons up to 1500 nucleotides⁶ have also been reported."

In the Fig. 2 caption, "(*Bsu* or *Sau*)" should be removed after "recombinase" and inserted after "polymerase". The corrected passage should read as follows: "The recombinase disassembles from the nucleoprotein filament once the strand exchange is performed, and will be available for the next pair of primers. Next, the DNA polymerase (*Bsu* or *Sau*) extends from the 3' end of primers."

In section 2.5, ref. 76 is not relevant as it is the same as ref. 77, and should be disregarded. The respective passage should read as follows: "However, several research groups have studied RPA reaction temperatures that lie outside of the recommended range.^{38,44,45,60,62–75,77,78} The largest temperature range was tested between 15 °C and 50 °C;^{62,64,69,70,77} and results indicated the marginal reaction temperature to produce a positive result should be greater than 30 °C.^{62–64,66,67,69,71,74,77}"

In section 2.5, ref. 63 was not interpreted correctly. The corrected text should read: "Moreover, Lillis *et al.*⁶³ showed that the ambient temperature also had an effect on RPA reaction: the RPA reaction was unstable if the ambient temperature was below 30 °C, even at extended reaction time."

In section 2.8, ref. 107 is not required and should be deleted. The corrected passage should read: "For the TwistAmp® nfo kit, however, two types of amplicons are generated... (note that only the dual-labelled product will generate a positive signal in the test zone of a lateral flow strip detection based on a sandwich assay).^{105,106,108}"

In section 2.8, the problems reported in ref. 117 were not sufficiently reflected in the original version of the review. Ref. 117 should be reported separately and the passage should read as follows: "As with lateral flow strip detection, direct usage of RPA amplicons is possible, but it is recommended to dilute the amplicons with the running buffer (*e.g.* 1/100 dilution) before running on the strip to (1) improve its wicking performance¹¹⁴ and (2) avoid "faint ghost band" effects.^{45,54,115,116} However, the dilution of the amplicon does not always prevent the appearance of a faint band, which can lead to specificity problems in the assay.¹¹⁷"

In section 3.2, ref. 166 was not cited correctly. The corrected version should read: "Results suggest that electrochemical detection could be up to 10-fold more sensitive than optical detection (by enzyme linked oligonucleotide assay).¹⁶⁶"

In section 3.2, a reference was not provided for the sensitivity of the GeneXpert MTB/RIF assay. A reference should be added to the end of the following passage: "This ruthenium compound-based electrochemical detection achieved 11 CFU mL⁻¹ of

^aLaboratory for MEMS Applications, IMTEK – Department of Microsystems Engineering, University of Freiburg, Georges-Köhler-Allee 103, 79110 Freiburg, Germany. E-mail: Felix.von.Stetten@Hahn-Schickard.de; Tel: +49 761 203-73243

^bInflammation and Healing Research Cluster, Genecology Research Centre, School of Science and Engineering, University of the Sunshine Coast, Qld, Australia. E-mail: jmacdon1@usc.edu.au; Tel: +61 7 5456 5944

^cDivision of Experimental Therapeutics, Columbia University, New York, NY, USA. E-mail: jm2236@columbia.edu

^dHahn-Schickard, Georges-Köhler-Allee 103, 79110 Freiburg, Germany



Mycobacterium tuberculosis analytical sensitivity, which is even more sensitive than the GeneXpert MTB/RIF (Cepheid Inc.) detection (a World Health Organisation recommended tuberculosis diagnostic system that employs PCR real-time fluorescent detection; 131 CFU mL⁻¹)." The added reference is shown below as ref. 1.

In section 3.4, ref. 113 was not cited correctly. Instead of *Mycobacterium bovis*, *Mycobacterium tuberculosis* was used for demonstration. The corrected sentence should read: "Liu *et al.*¹¹³ demonstrated a duplex detection of *IS6110* and *IS1081* insertion sequences of *Mycobacterium tuberculosis* using RPA-SMR assay, and achieved 3.2 and 12 genomic DNA copies per reaction analytical sensitivity respectively."

In section 4.1, ref. 182 and 183 were not cited precisely. The corrected version of the text should read: "The "microcliff" structured microchip demonstrated by Yeh *et al.* encased 200 to 1500 wells (30–100 nL per well),¹⁸² and 224 wells (100 nL per well),¹⁸³ which allowed detection of 10³–10⁵ and 10–10⁵ copies per μL of MRSA DNA, respectively."

In section 4.2, ref. 194 should be deleted after the following sentence: "For the latter, one demonstration is on the digital video disk (DVD) by Maquieira research group, and the resulting signals can be detected by a DVD player (Fig. 12B).^{188,191}"

Ref. 101 in the original article was incorrect and should be replaced with the correct reference, shown below as ref. 2.

Ref. 107 was not cited in the original article and should be disregarded.





Table 5 RPA literature describing clinical/field trials

Analyte(s)	Detection method	Limit of detection	Clinical/field sample(s)	Clinical sensitivity	Clinical specificity	Benchmark method	Limit of detection of benchmark method	Clinical sensitivity of benchmark method compared to RPA	Clinical specificity of benchmark method compared to RPA	Ref.
Nucleocapsid (N) gene of bovine coronavirus	Real-time fluorescent detection	10 to 100 RNA copies (19 RNA copies by probit analysis)	16 fecal and 14 nasal swab specimens collected from cattle showing intestinal and/or respiratory manifestations	100%	100%	Real-time RT ² -PCR	1000 RNA copies	The same	The same	125
Chlamydia trachomatis CDS2 gene	Lateral flow strip detection	5–12 pathogens/reaction	70 self-collected first void morning urine samples from young adults (19 males and 51 females)	83%	100%	Roche Cobas AmpliCor CT assay	—	Higher	The same	92
cAMP factor (<i>cfb</i>) gene of group B streptococci	Real-time fluorescent detection	98 genome copies	50 vaginal/anal samples collected from women	96%	100%	Real-time PCR	—	Higher	The same	126
DNA target sequence specific to <i>Cryptosporidium</i> spp.	Lateral flow strip detection	100 oocysts per mL stool	A total of 10 human stool samples clinically verified to contain cryptosporidium by a reference laboratory and 11 stool samples from healthy volunteers presumed to be uninfected	100%	100%	Real-time PCR	—	Lower	The same	127
5'-Untranslated region of Yellow fever virus (YFV)	Real-time fluorescent detection on the tube scanner	44 genomic copies/reaction in YFV RNA extracts; 21 genomic copies/reaction of YFV-spiked human plasma samples	34 samples of monospecific pools of wild-caught mosquitoes collected from Kedougou, southern Senegal	80%	100%	Real-time RT ² -PCR	8 genomic copies/reaction in YFV RNA extracts	Higher	The same	117
<i>IS6110</i> gene of <i>Mycobacterium tuberculosis</i> (MTB)	Real-time fluorescent detection	6.25 fg	27 RNA samples of mosquito pools	71.4%	100%	Culture	—	Higher	The same	128
<i>IS1081</i> gene of <i>Mycobacterium tuberculosis</i>	Real-time fluorescent detection	20 fg	121 specimens including induced and expectorated sputum (<i>n</i> = 119) and respiratory washes (bronchial and tracheal, <i>n</i> = 2) collected from a total of 101 tuberculosis suspect cases (no more than 3 specimens/individual were tested)	87.5%	95.4%	Culture	—	Higher	Higher	129
<i>Giardia</i> beta giardin gene	Lateral flow strip detection	10 ³ –10 ^{3.5} cysts per mL of stool	104 clinical stool samples	73%	96%	Real-time PCR	10 ^{3.5} cysts per mL of stool	Higher	Higher	129

Table 5 (Contd.)

Analyte(s)	Detection method	Limit of detection	Clinical/field sample(s)	Clinical sensitivity	Clinical specificity	Benchmark method	Limit of detection of benchmark method	Clinical sensitivity of benchmark method compared to RPA	Clinical specificity of benchmark method compared to RPA	Ref.
<i>ISG110</i> gene of <i>Mycobacterium tuberculosis</i>	Real-time photonic detection	10 ⁻⁶ -Fold diluted MTB sample	42 clinical samples including 13 smear and culture positive samples and 22 smear and culture negative samples	86%	95%	Real-time PCR	—	Higher	Higher	130 ^b
A highly conserved 3'-untranslated region that cover DENV 1-4	Real-time fluorescent detection	DENV serotype 1: 237 RNA copies; DENV serotype 2: 618 RNA copies; DENV serotype 3: 363 RNA copies; DENV serotype 4: 383 RNA copies	Inactivated DENV 1-4 spiked plasma and 31 DENV positive samples in Kedougou region in Senegal RNA of 90 plasma samples extracted and tested between 2012-2013 by RT ⁻ -PCR in Bangkok (Thailand)	98%	—	Real-time RT ⁻ -PCR	—	Higher	—	131
47 kDa gene sequence from the Karp strain of <i>Orientia tsutsugamushi</i> (47-RPA) and the 17 kDa gene sequence from the Wilmington strain of <i>Rickettsia typhi</i>	Lateral flow strip detection	53 DNA copies/reaction 17 kDa gene: 20 copies/reaction	10 positive and 10 negative human samples	80%	100%	Real-time PCR	47 kDa gene: 10 DNA copies/reaction 17 kDa gene: 6 DNA copies/reaction	Higher	Higher	95
Ribosomal 18S DNA of <i>Entamoeba histolytica</i>	Lateral flow strip detection	2.5 fg from serial dilutions of pure DNA extracted from parasites; 40 parasites from spiked stool sample	32 samples of DNA extracted from clinical samples	100%	100%	Real-time PCR	2.5 fg from serial dilutions of pure DNA extracted from parasites	The same	The same	132
A sequence designed based on ITS sequences of the <i>Madurella mycetomatis</i> type strain CBS 109801	Gel electrophoresis detection	0.23 ng of DNA	12 patient biopsy specimens	100%	100%	Conventional PCR	—	The same	The same	133
Ebola virus (EBOV) nucleocapsid sequence	Real-time fluorescent detection	5 genomic copies/reaction of a molecular RNA standard; 15 genomic copies/reaction in EBOV-spiked human plasma samples	928 post-mortem swab samples	100%	100%	Real-time RT ⁻ -PCR	—	The same	The same	134





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Orf virus (ORFV) DNA polymerase gene segments	Real-time fluorescent detection	100 DNA copies	22 samples collected from suspected cases of Orf, 8 nasal swabs collected from experimentally infected sheep and 5 samples obtained from healthy goats	86%	100%	Real-time PCR	—	Higher	The same	135
Leader peptidase A (<i>LepA</i>) gene of <i>Streptococcus pneumoniae</i>	Real-time fluorescent detection	4.1 genome equivalents/reaction	15 blood samples including 11 confirmed culture positive and 4 confirmed culture negative for <i>Streptococcus pneumoniae</i>	100%	100%	Real-time PCR	5.1 genome equivalents/reaction	The same	The same	97
Orf virus (ORFV) DNA polymerase gene segments	Lateral flow strip detection	80 copies/reaction of DNA plasmid	24 ORFV-spiked tissues samples, 53 samples collected from goats with suspected ORFV infection, 8 nasal swabs samples and 5 tissues samples from healthy goats	100%	100%	Real-time PCR	—	The same	The same	64
<i>Leishmania donovani</i> (LD) kinetoplast minicircle DNA	Real-time fluorescent detection	100 DNA copies applying the LD DNA linearised plasmid; 1 genomic DNA copy	96 buffy coats and skin biopsies collected from visceral leishmaniasis, asymptomatic and post-kala-azar dermal leishmaniasis	100%	100%	Real-time PCR	—	The same	The same	121
Highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) NSP2 gene	Real-time fluorescent detection	70 RNA copies/reaction	68 tissue samples and 10 serum samples collected from suspected pigs of HP-PRRSV, 35 serum samples and 12 tissue samples collected from healthy pigs	97.6%	100%	Real-time RT ² -PCR	—	Higher	The same	136
100% conserved sequence of a major capsid protein gene of all cyprinid herpesvirus 3 strains	Gel electrophoresis detection	10 copies of genomic DNA	12 confirmed latently infected fish and 1 confirmed uninfected fish	100%	100%	Real-time PCR	—	Lower	The same	66
cAMP factor (cfb) gene of group B streptococci	Real-time fluorescent detection	6.25–12.5 genome equivalents	124 clinical samples	100%	100%	Real-time PCR	3.1–6.25 genome equivalents	The same	The same	137
Non-structure protein 1 (nsP1) of Chikungunya virus (CHIKV)	Real-time fluorescent detection	80 genome copies of extracted RNA from CHIKV isolate LR strain	58 suspect Chikungunya fever cases	100%	100%	Real-time PCR	80 genome copies of extracted RNA from CHIKV isolate LR strain	The same	The same	87
A sequence designed in NS2A region conserved among all Zika virus lineages	Real-time fluorescent detection	21 RNA copies	25 positive and 9 negative urine samples collected during the Zika virus epidemic in Tuparetama, Brazil	92%	100%	Real-time RT ² -PCR	—	Higher	The same	138

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G-protein-coupled chemokine receptor (GPCR) gene of lumpy skin disease virus (LSDV)	Real-time fluorescent detection	100 DNA copies (179 DNA copies by probit analysis)	12 negative skin samples and 22 skin nodules of suspected LSDV-infected cattle collected during the summer of 2012 in Dakahlia Governorate, Egypt	100%	100%	Real-time PCR	37 DNA copies	The same	The same	139
<i>IS900</i> gene of <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> (MAP)	Real-time fluorescent detection	16 plasmid copies per μL ; 500 fg genomic DNA/reaction	Archived DNA of MAP positive blood ($n = 14$), sperm ($n = 18$), faecal ($n = 12$) and tissue ($n = 4$) samples and 20 MAP-negative faecal samples	89.5%	—	Real-time PCR	1 plasmid copies per μL ; 50 fg genomic DNA/reaction	Higher	—	140
T1E4 gene of prostate cancer	Real-time fluorescent detection	1000 RNA copies	9 urine samples obtained from prostate cancer and 2 urine samples from healthy individuals	90%	100%	Real-time RT ² -PCR	—	The same	The same	141
NS1 gene of porcine parvovirus (PPV)	Real-time fluorescent detection	300 DNA copies	101 clinical tissue samples (serum, liver, kidney, lymph node, spleen and duodenum) collected from pig farms with suspected cases of PPV in Gansu province, China, and 27 clinical samples (serum, kidney and duodenum) collected from healthy pigs	94.4%	100%	Real-time PCR	—	Higher	The same	54
Nucleocapsid gene of type 2 porcine reproductive and respiratory syndrome virus (PRRSV)	Real-time fluorescent detection	100 RNA copies (690 RNA copies by probit analysis)	60 clinical samples (lymph node, lung, spleen and liver) collected from diseased pigs suspected of having PRRS from 5 pigs farms in Hebei province, China from 2015–2016	—	—	Real-time RT ² -PCR	100 RNA copies	—	—	142
Cytochrome b gene of <i>Theileria annulata</i>	Lateral flow strip detection	2 pg genomic DNA	17 anticoagulated blood samples collected from tropical theileriosis endemic areas in Gansu province, China	—	—	Real-time PCR	—	—	—	67
pirA-like gene of <i>Vibrio owensii</i>	Real-time fluorescent detection	2 plasmid copies (2.84 plasmid copies by probit analysis)	138 clinical shrimp obtained from immersion bioassay, including 70 shrimp acute hepatopancreatic necrosis disease (AHPND) infected shrimp and 68 non-AHPND infected shrimp	100%	100%	Real-time PCR	—	Lower	Lower	143



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rRNA gene of <i>Fasciola hepatica</i>	Gel electrophoresis detection	1.6 pg μL^{-1} DNA copies	102 human stool samples selected from banked specimens	87.8%	100%	Real-time PCR	1.6 pg μL^{-1} DNA copies	Lower	The same	144
	Lateral flow strip detection	1.0 pg μL^{-1} DNA copies		95.2%	90.4%			Lower	Higher	
N gene of pest des petits ruminants virus (PPRV)	Real-time fluorescent detection	100 plasmid copies	32 clinical samples collected from suspected cases of PPRV in Gansu province, China and 5 samples obtained from healthy sheep	90%	100%	Real-time RT ² -PCR	10 plasmid copies	Higher	The same	145
	Lateral flow strip detection	150 plasmid copies		90%	100%			Higher	The same	
ITS2 gene of <i>Phytophthora infestans</i>	Real-time fluorescent detection	50 fg μL^{-1} of genomic DNA	24 potato leaf samples collected from fields with and without visible symptoms of late blight infections in New Brunswick and Quebec provinces, Canada, respectively	33.3%	100%	LAMP	50 fg μL^{-1} of genomic DNA	Higher	Lower	146
	Lateral flow strip detection	100 plasmid copies		100%	100%			The same	The same	
ORF2 gene of porcine circovirus type 2 (PCV2)	Real-time fluorescent detection	100 plasmid copies	65 clinical samples (spleen, inguinal lymph node, tonsil, lung and serum) collected from suspected PCV2 infection pigs from 8 pig farms in Shandong province, China; 37 clinical samples (inguinal lymph node, tonsil, lung and serum) collected from Gansu Province, China, and 10 PCV1 positive samples conserved in the laboratory	100%	100%	Real-time PCR	80 plasmid copies	The same	The same	69
	Lateral flow strip detection	100 plasmid copies		100%	100%			The same	The same	
gD gene of pseudorabies virus	Real-time fluorescent detection	100 DNA copies	76 clinical samples (tonsil, heart, spleen, lymph nodes, lung and serum) collected from pig farms in Shandong province, China, and 26 clinical samples (lymph nodes, tonsil and serum) collected from healthy pigs	93.3%	100%	Real-time PCR	—	Higher	The same	70
	Lateral flow strip detection	160 DNA copies		93.3%	100%			Higher	The same	
B1 gene of <i>Toxoplasma gondii</i>	Lateral flow strip detection	0.1 oocysts/reaction	35 soil samples and 15 water samples collected from parks, residential areas, schools and gutterways in Lanzhou city, Gansu rovince, China, during August 2016	100%	100%	Nested PCR	1 oocyst/reaction	The same	The same	71



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RNA transcript of TMPRSS2:ERG (a fusion gene for prostate cancer)	RPA flocculation assay	10 ⁵ RNA copies	Clinical urine specimens from 10 metastatic castration-resistant promising prostate cancer patients and 5 healthy control patients	70%	100%	Conventional RT ² -PCR	—	The same	The same	101
VP2 gene of porcine parvovirus	Real-time fluorescent detection	100 DNA copies (103 DNA copies by probit analysis)	115 clinical samples (lymph node, lung, spleen, kidney and duodenum collected from pigs with reproductive disorders, diarrhea or respiratory disease in Hebei province, China from 2014 to 2016)	100%	100%	Real-time PCR	100 DNA copies	The same	The same	147
G-protein-coupled chemokine receptor (GPCR) gene of <i>Capripoxvirus</i>	Real-time fluorescent detection Lateral flow strip detection	300 plasmid copies 300 plasmid copies	107 clinical samples (liver, lung, kidney, spleen, skin and blood) collected from 14 suspected sheep and 6 suspected goats in Gansu province which were characterised by pyrexia, excessive salivation and generalised pox lesions in the skin during the period of October 2014 to August 2015	97%	100%	Real-time PCR	—	Higher	The same	148
Nucleocapsid protein gene of canine distemper virus	Real-time fluorescent detection	9.4 RNA copies (31.8 RNA copies by probit analysis)	32 nasal/oropharyngeal swabs collected from 20 dogs of both sexes (various breeds and ages) from the animal hospital of Agricultural University of Hebei and 12 raccoon dogs from the farms in Hebei Province, China from 2014 to 2016	100%	100%	Real-time RT ² -PCR	94 RNA copies	The same	The same	149
<i>imp</i> gene of <i>Candidatus</i> <i>Phytoplasma oryzae</i>	Real-time fluorescent detection Lateral flow strip detection	1–10 plasmid copies 10–100 plasmid copies	66 Napier grass samples from various geographical locations in western Kenya	100%	57.1%	Real-time PCR	—	Lower	The same	79



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<i>imp</i> gene of <i>Candidatus</i> Phytoplasma mali	Real-time fluorescent detection Lateral flow strip detection	10 copies of cloned plasmid 10 copies of cloned plasmid	38 roots of field samples from apple (<i>Malus domestica</i>) trees collected in autumn 2014, in spring 2015 and in June 2016 in private orchards or in the experimental field of the Institute for fruit growing in Samochvalovich, Belarus	100% 100%	100% 100%	Real-time PCR	—	The same	The same	72
N gene of rabies	Real-time fluorescent detection	1000 RNA copies per μ L of strains SAD B19, Bobcat USA and Kelev	A panel of RNA from 33 field samples	97%	—	Real-time PCR	1 RNA copies per μ L of strains SAD B19, Bobcat USA and Kelev	Higher	—	150
<i>KRAS</i> oncogenic mutation gene G12D on Exon 12	Real-time silicon photonic microring-based detection	1% to 100% of the mutant cells	70 frozen tissues samples from colorectal cancer patients in Bio-Resource Center, including 24 samples with the G12D mutation (34.3%), 26 samples with G13D mutation (37.1%) and 20 samples with no mutation (28.6%)	100%	100%	Conventional PCR	30% to 100% of the mutant cells	Lower	The same	151
<i>KRAS</i> oncogenic mutation genes G13D on Exon 13	Real-time fluorescent detection	500 RNA copies (251 RNA copies by probit analysis)	21 extracted patient sera samples obtained in relation to outbreaks of CCHFV in 2013–2015 in Tajikistan	88%	100%	Real-time PCR	—	Higher	The same	152
A consensus region that covers all 7 S-segment clades of Crimean-Congo Hemorrhagic fever virus (CCHFV)	Real-time fluorescent detection	10 copies of recombinant plasmid	91 fecal swab samples collected from the dogs from 2012 to 2016	100%	100%	Real-time PCR	10 copies of recombinant plasmid	The same	The same	153
Canine parvovirus 2 (CPV-2) nucleocapsid protein gene	Lateral flow strip detection	8 plasmid copies/reaction (corresponding to 24 RNA copies)	104 clinical blood specimens and 24 tissue samples including 16 lung tissue specimens, 8 lymph gland specimens collected from suspected dairy cattle cases of BEFV infections in eastern China	97.89%	90.91%	Real-time PCR	—	Higher	Higher	74



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<i>IS900</i> gene of <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>	Lateral flow strip detection	8 plasmid copies/reaction	320 individual fecal samples collected between September 2016 and September 2017 from 10 different dairy farms located in 10 distinct geographic regions of Shandong province, China	100%	97.63%	Real-time PCR	8 plasmid copies/reaction	The same	Higher	77
<i>Fho</i> FSC771 hypothetical protein gene of <i>Francisella noatunensis</i> subsp. <i>Orientalis</i>	Real-time fluorescent detection	10 plasmid copies (15 plasmid copies by probit analysis)	Samples of spleen ($n = 78$), head kidney ($n = 78$) and water ($n = 5$)	100%	84.89%	Real-time PCR	10 plasmid copies (11 plasmid copies by probit analysis)	The same	Higher	154
VP1 gene of Enterovirus 71 subgenotype C4 (EV71-C4)	Real-time fluorescent detection	3.767 log ₁₀ genomic copies (LGC)	Stool samples ($n = 44$) collected in 2017 by Shenzhen Center for Disease Control and Prevention	100%	100%	Real-time PCR	2.026 log ₁₀ genomic copies (LGC)	The same	The same	155
56 kDa gene of a Karp-like strain of <i>Orientia tsutsugamushi</i>	Lateral flow strip detection	10 copies (recombinant plasmid); 12 copies of genomic DNA	Stool samples ($n = 134$) collected from patients with suspected hand-foot-mouth disease at the pediatrics department of Zhujiang Hospital (Southern Medical University, Guangzhou, China) in 2009	89.5%	100%	Real-time PCR	Lower	Lower	The same	156
23S rRNA gene of <i>Coxiella burnetii</i>	Lateral flow strip detection	10 copies (recombinant plasmid); 7 copies of genomic DNA	62 animal (including <i>Apodemus agrarius</i> , <i>Rattus norvegicus</i> , <i>Microtus fortis</i> and <i>Neomys fodiens</i>) organ samples including 5 infected animals trapped in the wild, 2 infected in the laboratory and 55 uninfected animals trapped in the wild	100%	100%	Real-time PCR	12 copies of genomic DNA	The same	The same	157

^a RT: reverse transcription. ^b Clinical sensitivity and specificity were recalculated using the ESI.

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