BDBV specific PCR (PHAS)_V1 (2026-05-22)

Extraction:

Sample Inactivation (each laboratory should conduct their own risk assessment):

Mix 200 µl of sample and 600 µl of TRIzol reagent and vortex thoroughly to ensure complete mixing. Incubate at room temperature for 5 minutes.

Phase Separation (BSL-2):

1. Add 160 µl Chloroform, vortex thoroughly to ensure complete mixing and incubate at room temperature for 3 minutes.
2. Centrifuge the tube at 4°C 12000 x g (RCF) for 15 minutes.
3. After Centrifugation, a phase separation will form.
4. Transfer 200 µl of the upper aqueous phase to a new tube without disturbing the interphase.
5. Continue with automated MagLEAD Extraction or use QIAamp Viral Mini kit for manual extraction according to manufacturer's instruction.

Automated MagLEAD Extraction:

According to manufacturer's instruction with elution volume of 50 µl.

One step Real-time RT-PCR for detection of Ebola Bundibugyo virus:

The BDBV RT-PCR assays are carried out in 25 µL reaction mixtures containing TaqMan Fast Virus 1-step Master Mix (Applied Biosystems, Thermo Fisher Scientific), 5 µL template RNA, DNase/RNase-free H2O (Life Technologies, Thermo Fisher Scientific), 0.9 µM of each primer (0.3 µM BDBV NP-F), and 0.2 µM of TaqMan probes (Applied Biosystems).

Amplification and detection of the amplicon is performed in a StepOne Plus real-time PCR system (Applied Biosystems).

To ensure adequate RNA extraction, the presence of Beta-actin mRNA in clinical samples is analysed using a TaqMan gene expression assay (Applied Biosystems).

Primer and probe sequences for EBOV L (Bundibugyo) and EBOV NP (Bundibugyo):

Target BDBV L	**Sequence (5'–3')**
BDBV_F	[TCATTAGTTGAGATCAAGACTGGTTTC]
BDBV_R	[GGAAAGAACTGTGATGCATTGATTAT]
BDBV/Tai F_P	[FAM- CCCATTACCGCAGATC -MGB (NFQ)]
Target BDBV NP	Sequence (5'–3')
BDBV_NP_F	[CCTGTCTGGAGAAGGTTCAACG]
BDBV_NP_R	[TCGGATATTGAATCAGACCTTGTTC]
BDBV_NP_P	[FAM- ACAAATCCAAGTGCACGC -MGB (NFQ)]

Reaction mixture EBOV L BDBV:

Master mix for 25 µL reaction	Final konc.	µL /reaction
TaqMan FAST Virus 1-step MM (4x)	1x	6,25
BDBV_F (10 µM)	0,9 µM	2,25
BDBV_R (10 µM)	0,9 µM	2,25
Bundi/Tai F_P (10 µM)	0,2 µM	0,5
H_2O	NA	8,75
Volume	–	20
RNA-template	–	5

Reaction mixture EBOV NP BDBV:

Master mix for 25 µL reaction	Final konc.	µL /reaction
TaqMan FAST Virus 1-step MM (4x)	1x	6,25
BDBV_F (10 µM)	0,3 µM	0,75
BDBV_R (10 µM)	0,9 µM	2,25
Bundi/Tai F_P (10 µM)	0,2 µM	0,5
H_2O	NA	10,25
Volyme	–	20
RNA-template	–	5

Positive control:

Bundibugyo vRNA virus (5 µl/PCR reaction) for L PCR and Bundibugyo IVT RNA for NP PCR

The cycling profile:

50°C for 5 min; 95°C for 20 s; 45 cycles of 95°C for 3 s and 60°C for 30 s.

Samples are considered positive if target amplification is recorded within 40 cycles (cycle threshold (Cq) ≤ 40). The baseline and threshold were set using the auto-baseline and auto-threshold features in the StepOne software (Applied Biosystems).

Performance: The BDBV L and NP real-time RT-PCR has been evaluated with:

BDBV viral RNA (PHE) in 1:5 dilutions, detected to final dilution step at Ct-values of 38 BDBV L qPCR and 36 BDBV NP qPCR.

- Negative serum (n = 2)
- Negative EDTA whole blood (n = 2)
- RNA extracted from EBOV Zaire, EBOV SUDV, EBOV Thai Forest virus.

None of the above negative samples or closely related EBOV (Zaire, SUDV and Thai Forest) resulted in non-specific amplification

Total BDBV RNA (source: Bundibugyo virus RNA from the current outbreak, Charité) to an approximate resulting RT-PCR Ct value of 29 (Altona kit), corresponding to ca. 2000 copies/µL of viral RNA.

- A dilution series BDBV RNA was analysed in duplicate by above-mentioned PCR targeting BDBV L and NP, ranging from undiluted to a 1:100 000 dilution. Positive amplification was observed up to 1:1000 dilution for both BDBV L and NP resulting in Ct values of 38,7 (BDBV L PCR) and 37,6 (BDBV NP PCR). The detected Ct values for undiluted RNA were 27,7 and 27,8 respectively.

- Therefore, the assay demonstrated detection of RNA down to approximately 10 copies per PCR reaction, corresponding to 2 copies/µL. Based on original data provided and these data, the practical limit of detection (LoD) of the both PCR assays is estimated to be around 10 RNA copies per reaction.