Protocol for Real-Time RT-PCR of EboZaire DUO with P&P

For RUO (Research Use Only)

Caution: The vials containing the primers and probe mix (P&P) should be stored after reception at -20°C in the dark. Stable at least 6 months in the described conditions.

NB: Probe: FAM-TAMRA

Development and evaluation of a duo Zaire ebolavirus Real-Time RT-PCR assay targeting two regions within the genome. Thirion L, Charrel R. N., Boehmann Y., Corcostegui I., Raoul H., Lamballerie X. *Microorganisms*, 2019, 7 (12), art. 652 [15 p.]

Reagents with which the assay has been validated at UVE (Unité des Virus Emergents, Marseille, France):

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit, ThermoFischer ref 11732-088
- Water molecular grade
- IVT RNA Ebola Z specific

1. Kit contents:

Nom	Number of vials	Number of assays/vial
QC_Lyoph-P&P EboZaire Duo		8+1

Table 1. List of products in the kit

1. Rehydration of Lyoph-P&P before use (Table 1)

- Write the date on the vial before opening. Lyoph-P&P is respudended as described:
 - Add 63 µl of Water molecular grade
 - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 30μL-volume
 - Rehydrated P&P must be incubated at room temperature for 10 min after which
 - A second step of 10 times multiple pipetting must be done.

WARNING: These steps are critical to ensure adequate homogenization

Number of tests/vial	9
H2O (µL)	63

Table 2. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 7 days

^{*} this volume is adapted to the SuperScript™ III Platinum™ One-Step qRT-PCR Kit and can vary depending upon the RT-PCR kit

2. Preparation of the reaction mix

MasterMix	25μL Single rxn, μL
2X Reaction mix*	12,5
Rehydrated Primers and probe P&P**	7
SSIII/Taq Enzyme Mix*	0,5
Total	20
Template RNA	5

^{*,} ThermoFischer / Invitrogen: SuperscriptIII One-Step RT-PCR system with Platinum Taq DNA Polymerase

3. Cycling program and RT-PCR reaction

1:50°C - 15min

2:95°C - 2min

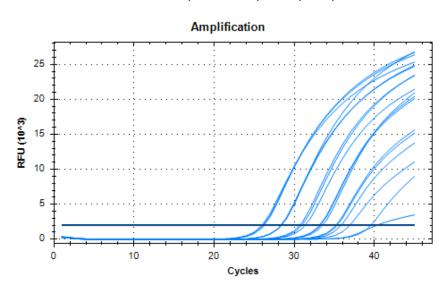
3:95°C-15sec

4:60°C - 30sec - Plate read

5: GOTO3, 45 more time

IVT Ebola Z 10-9	
Mean Ct : 26.11	

Result obtain with DuoEboZ specific assay and $5\mu L$ of positive control



^{**,} as indicated in Table 2.