Protocol RSV laboratory diagnosis: positive control (gblock – RSV)

08 March 2023

Laboratory Protocol

1) Prepare in the clean room:

a) One tube with 35uL TE (to resuspend stock solution). Label it as "Tube 0". We will use 25uL.

b) One tube with 990uL TE (to do the first dilution, 10E-2). Label it as "Tube B".

c) Seven tubes with 90uL TE (to titration). Label it as "Tube3", "Tube4",… "Tube 9" and add the date.

Note: For resuspension, using TE pH 7,5 or 8,0 is preferable. If unavailable, molecular biology-grade water can be used.

2) In the extraction room

a) Resuspension of gblock to 10ng/uL

1) Spin the positive control tube (the original tube from the manufacturer) for 3000g/1min, to ensure that all particles go down to the bottom of the tube.
2) Add 25uL of TE from **Tube 0** in the positive control tube. Close the tube tightly, label it "Tube A", and add the date.
3) Vortex briefly
4) Incubate for 50°C / 20 minutes
5) Vortex briefly again
6) Final Spin
7) Discard Tube 0

3) First Dilution (1:100)

i) Clean a BSC or a workstation containing UV light and put only the stuff you need inside: Eppendorf rack, pipette (p20), tips for loading 10uL, discarding bin and a hypochlorite solution for surface decontamination.

ii) Inside the BSC or a workstation with UV light, set a pipette to 10uL, and with a new tip, pipette up and down five times (5x) inside Tube A (the original tube from the manufacturer) to homogenate.

NOTE: Be careful. This tube contains an enormous amount of DNA and can indefinitely contaminate pipettes, surfaces, lab coats, etc. Do not spill.

iii) After the homogenization, using the same tip, take 10uL from Tube A and add inside Tube B, containing 990uL of TE.

iv) Pipette up and down five times (5x) inside Tube B to homogenize. Leave all the volume (990uL, TE + 10uL= 1000uL, [10E-2]) in the last time.

v) Discard this void tip in the bin. Do not touch the tip.

vi) Take a new tip and pipette up and down five times (5x) inside Tube B to homogenize.

4) Titration (dilutions 10E-3 to 10E-9)

vii) After the homogenization, using the same tip, take 10uL from Tube B and add inside Tube 3, containing 90uL of TE.

viii) Pipette up and down five times (5x) inside Tube 3 to homogenate. Leave all the volume (90uL, TE + 10uL=100uL [10E-3]) in the last time.

ix) Discard this void tip in the bin. Do not touch the tip.
x) Take a new tip and pipette up and down five times (5x) inside Tube 1.
xi) After homogenizing, with this new tip, take 10uL from Tube 3 to Tube 4, containing 90uL of TE.
xii) Pipette up and down five times (5x) inside Tube 4 to homogenate. Leave all the volume the last time.
xiii) Discard the void tip in the bin.
xiv) Take a new tip and pipette up and down five times (5x) inside Tube 4.
xv) After homogenizing, with this new tip, take 10uL from Tube 4 to Tube 5, containing 90uL of TE.
xvi) Pipette up and down five times (5x) inside Tube 5 to homogenate. Leave all the volume the last time.
xvii) Proceed as described from "v" to "ix" and repeat it, changing the tips when you take the 10uL to the following dilution, up to 10E-9 dilution.
xviii) Store the positive controls (Tube A, Tube B and dilution tubes after use) in a freezer -70°C, in a safe place.

5) Titration summary
   a) Take 10uL of Tube A (Stock Suspension), to tube B (990uL+10uL, [10E-2])
   b) Take 10uL of Tube B to Tube 3 (90uL+10uL, [10E-3])
   c) Take 10uL of Tube 3 to Tube 4 (90uL+10uL, [10E-4])
   d) Take 10uL of Tube 4 to Tube 5 (90uL+10uL, [10E-5])
   e) Take 10uL of Tube 5 to Tube 6 (90uL+10uL, [10E-6])
   f) Take 10uL of Tube 6 to Tube 7 (90uL+10uL, [10E-7])
   g) Take 10uL of Tube 7 to Tube 8 (90uL+10uL, [10E-8])
   h) Take 10uL of Tube 8 to Tube 10 (90uL+10uL, [10E-9])

To finish, decontaminate the pipette, rack, and surfaces you used to dilute and resuspend the controls. Keep UV for 30min in the BSC or workstation after using it again.

RT-PCR

Perform the RT-PCR for RSV detection as indicated by the technical note: Respiratory Syncytial Virus (RSV) laboratory diagnosis from PAHO, using the 10E-4 to 10E-8 dilutions as templates. For more accuracy, use duplicates.

You will likely have Cts around 18 for 10E-5 and 36 for 10E-9. Choose the dilution that gives you Ct around 24-29 and use it as your positive control.

You can maintain it in the refrigerator for one to three months, observing the decay in the performance, or make aliquots of 10uL and keep at -20°C for a year.