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Isolation of RNA from blood

1. 500 µL of fresh blood put on eppendof 1.5 mL
2. Added 500 µL tri reagent, was pipetting
3. Incubated for 5 minutes in romm temperature
4. Added 100 µL chloroform
5. shake vigorously for 15 seconds
6. incubated for 10-15 minutes at room temperature
7. Centrifuged at 12,000 rpm 4 ° C for 15 minutes
8. Taken / transfer aqueous phase (in top layer) to another eppendof 1.5 mL
9. Added 250 µL isopropanol
10. Incubated for 5-10 minutes at room temperature
11. Centrifuged 12000 rpm 4 ° C for 8 minutes , the supernatant was discarded , was obtained the white pellet
12. Added the Pellets with 500 µL 75 % ethanol
13. Centrifuged 7500 rpm 4 ° C for 5 minutes, the supernatant was discarded completely , was obtained RNA pellet
14. Briefly air-dry the RNA pellets for 3-5 minutes remain in the laminar
15. Added 20 µL of water free RNA
16. The results are stored at -80 ° C for RT RNA or PCR process , in part to determine the levels and purity using the NanoDrop spectrometer

    Note :
    a. All procedures must doing in the laminary Air flow
    b . All tools (eppendof , yellow type , blue type , white type of must have been in treatment with DEPC water treatment