Troubleshooting common challenges associated with SARS-CoV-2 diagnostic test establishment

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Algorithm and reporting

Screening Assay
E gene (+ EAV*)

Positive

Confirmation Assay
RdRp gene

Positive

Confirmed case

Negative

Indeterminate
Repeat assay with the extracted RNA as well as the different stored specimen aliquot.
In parallel collect further specimens and if necessary refer to a laboratory with greater experience of testing for SARS-CoV-2

Indeterminate

HOWEVER: Collect further specimens and repeat tests if clinical or epidemiological evidence is suggestive, or if initial specimen was of poor quality

Inconclusive
Collect further specimens and repeat tests if clinical or epidemiological evidence is suggestive, or if initial specimen was of poor quality

*EAV negative

NTCs and Neg controls should all be negative; if not = PCR failure/contamination
E gene Ct value distribution

Sample

Ct value

n = 68
RdRp gene Ct value distribution

$n = 33$

Sample

Ct value

39.41

20.15

n = 33
Matched E and RdRp Ct comparison

- Range: 0.7-2 Ct difference
- Generally E has a lower Ct

![](chart.png)

n = 33
Currently extremely high demand for SARS-CoV-2 real-time PCR kits

- Primer/probe quality is of huge concern, so treat these with extra precaution eg after resuspension and dilution, aliquot immediately into enough for one run

- Issues with contamination (reported by Australia, Europe, Hong Kong, US) leading to false positives – clear to detect as NTCs come up as positive as well; so please be wary
Alternate discussion topics

• **Multiplex**: eg two or more gene targets and/or extraction control
  • Reduces work load and need for subsequent confirmatory assay

• **Global reagent stocks** are in short supply with no guarantee for delivery from US and Europe to Africa; might be some options with China
  • In-house assay set-up
  • Primers, probes and enzyme can be locally sourced or sourced from other African companies would alleviate some of these issues
Questions from WhatsApp group
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• What is the guide for re-testing COVID-19 positive cases for discharge?
  • Must be PCR negative twice
  • But must self-isolate for 14 days

WHO interim guidance: Laboratory testing strategy recommendations for COVID-19
Questions from WhatsApp group

• What is the procedure for reconstitution of enzyme?
  • Please refer to “1-step RT-PCR Polymerase Mix” instructions that we included in the training packs:
    • Transfer the whole content of once vial of qRT-PCR probe reconstitution buffer to one vial of qRT-PCR mix (beads)
    • Mix well but do not vortex
Questions from WhatsApp group

• I would like to know the number of copies by ul of SARS-CoV-2 positive controls (E and RdRp genes).
  • I have contacted TIB Molbiol and will update when I have had a response
Laboratory-confirmed case by NAAT in areas with established COVID-19 virus circulation.

In areas where COVID-19 virus is widely spread a simpler algorithm might be adopted in which, for example, screening by rRT-PCR of a single discriminatory target is considered sufficient.
Questions from WhatsApp group

• Our repeat RdRp worked but the E and N gene positive samples turned out negative for the RdRp gene, is there some explanation?

• Did we not say during the training that the E gene is used for screening on the assumption that we currently do not have SARS-CoV in circulation. Would this need sequencing for confirmation if the RdRp is not coming out as expected.