NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

Division of the National Health Laboratory Service

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

Jinal N. Bhiman (PhD)
Centre for Respiratory Diseases and Meningitis
National Institute for Communicable Diseases

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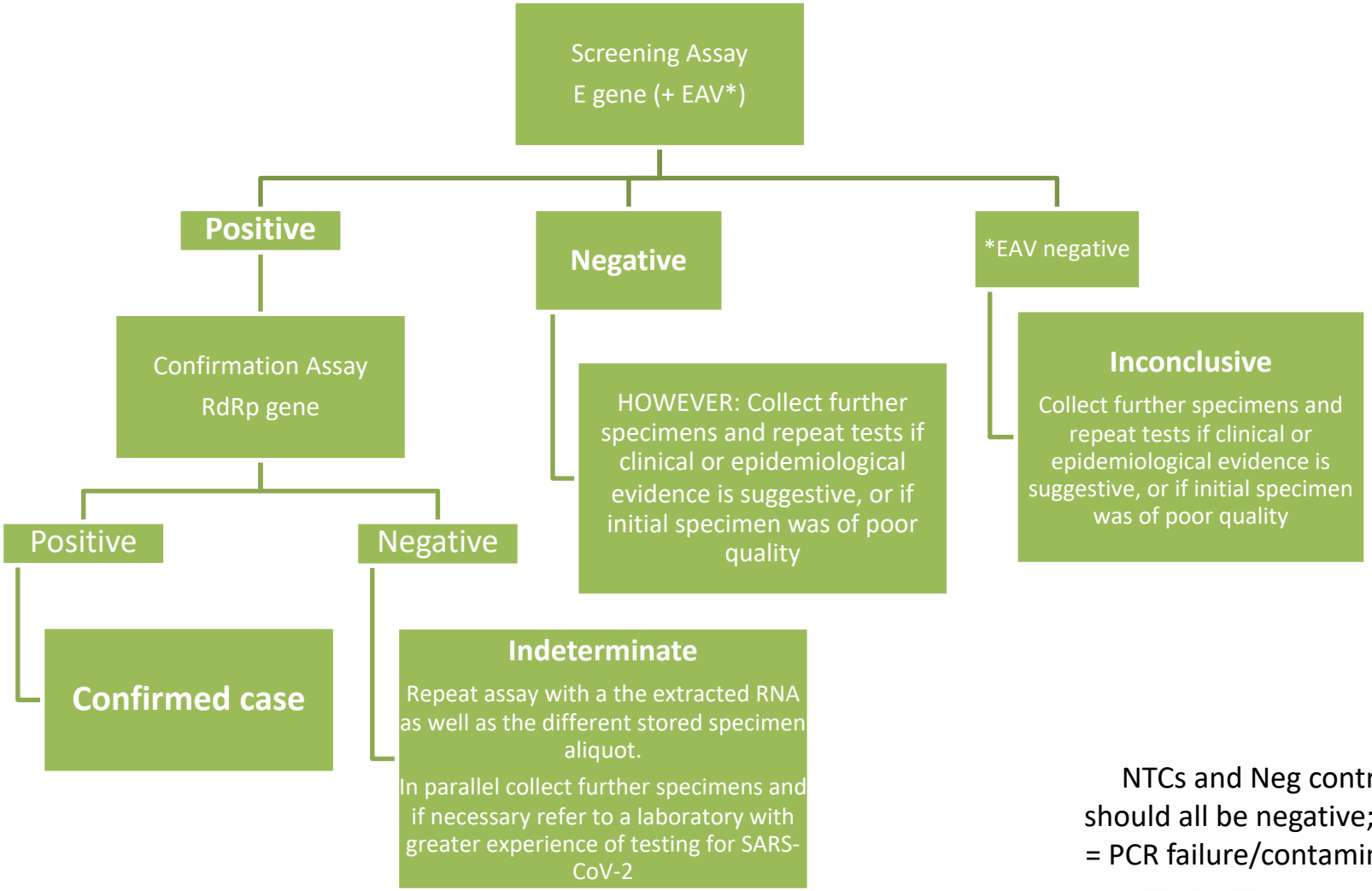
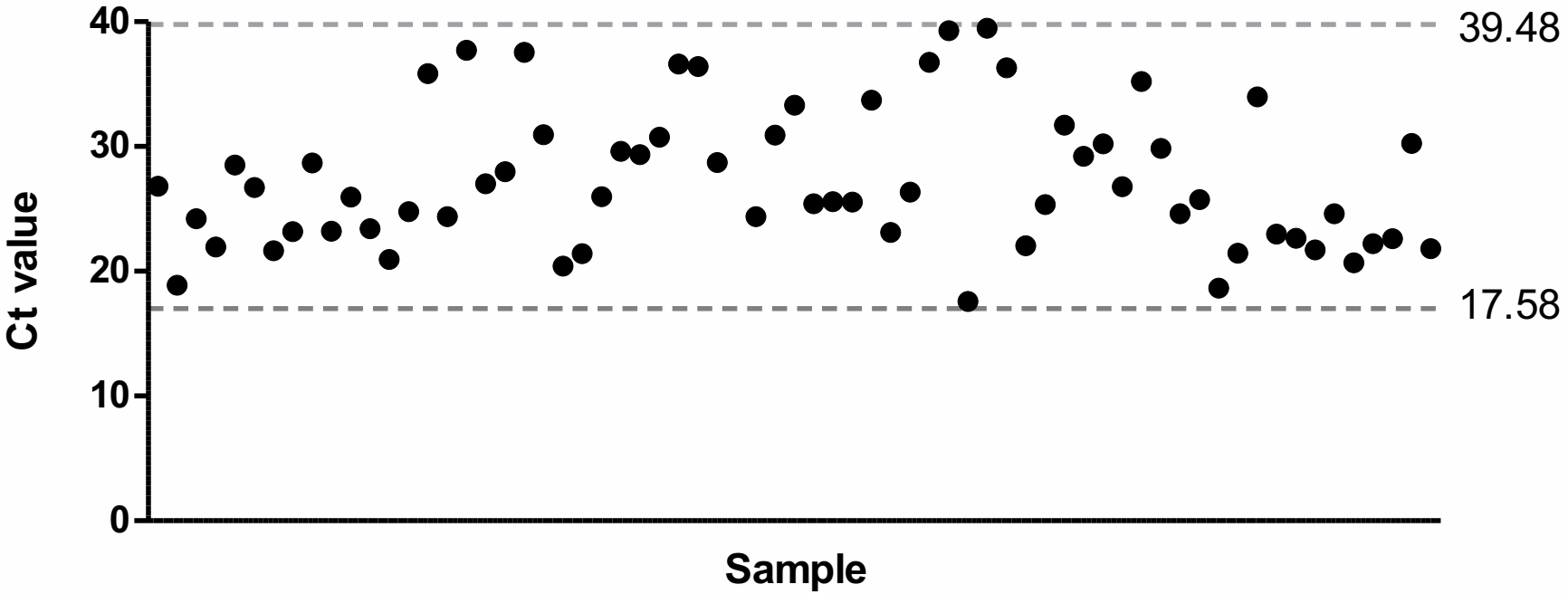
Algorithm and reporting



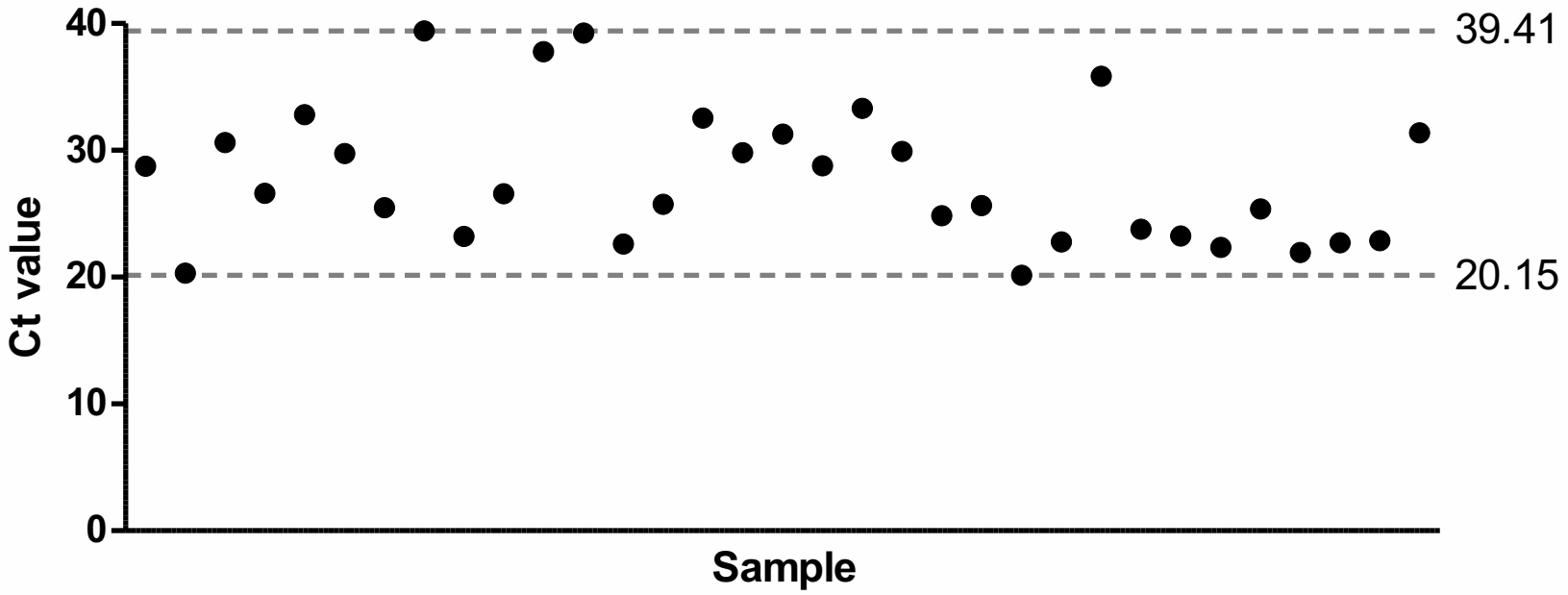
NTCs and Neg controls should all be negative; if not = PCR failure/contamination

E gene Ct value distribution



n = 68

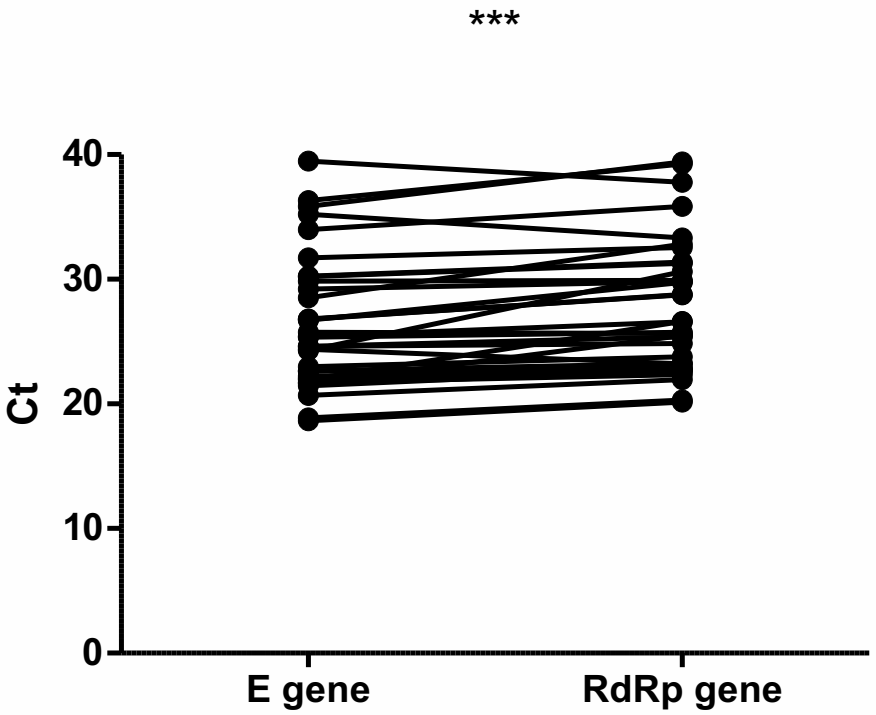
RdRp gene Ct value distribution



n = 33

Matched E and RdRp Ct comparison

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



n = 33

Kit/primer/probe quality

- 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

WHO recommendations

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.

WHO interim guidance : Laboratory testing for coronavirus disease (COVID-19) in suspected human cases
19 March 2020

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.