

**Repeat Testing Outcomes of Inconclusive SARS-CoV-2 RT-PCR
results in inpatients at Charlotte Maxeke Johannesburg Academic
Hospital (CMJAH)**



Student Name: Nombuso Precious Nxele BSc (Hons), MBChB, Dip HIV Man, DTM&H

Student Number: 2503547

Masters in Medicine (MMed) Virology Research Dissertation

23 August 2024

Supervisors:

Supervisor: Dr Zinhle Makatini BSc (Hons), MSc, MBChB, FCPATH Viro (SA), DTM&H, PhD

Co-supervisor: Dr Lerato Sikhosana MBChB, MPH, FCPATH Viro (SA)

Co-supervisor: Hloni Mthiyane BTEC, MSc

Co-supervisor: Dr Mokopi Moloi BSc (Hons), MSc, MBChB, FCPATH Viro (SA), DTM&H

Copyright © Dr Nombuso Nxele

All rights reserved.

Declaration

I declare that the dissertation hereby submitted to the University of Witwatersrand, Faculty of Health Sciences for the Degree in Masters of Medicine (Virology), has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

A handwritten signature in black ink, appearing to be 'M. M. M.', written in a cursive style.

10 March 2024

Dedication

I dedicate this work with gratitude to my late parents Mr JR and Mrs TA Nxele for instilling a culture of positive attitude and hard work in me. A special thank you to my children Phathisiwe and Khethokuhle Nxele, my number one fans for the sacrifices they made in allowing me to ensure that this work is a success. My deepest gratitude to my mentor and supervisor Dr Zinhle Makatini for her invaluable support and guidance. The experience I gained working with her is priceless. A final thank you to family and friends for the constant support and prayers throughout this journey.

Abstract

Background

With an analytical sensitivity of > 99% in samples harbouring viral loads between 500 – 5000 copies/ml, SARS-CoV-2 RT-PCR is regarded as the gold standard test method for the diagnosis of COVID-19. The reality of inconclusive results occurring, presents a laboratory diagnostic challenge that has an impact on patient management as well as infection and control preventative measures.

Methods

Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) Virology laboratory performed a total of 42790 SARS-CoV-2 RT PCR tests using the Allplex™ 2019-nCoV (Allplex; Seegene, Seoul, Korea) molecular assay on nasopharyngeal swabs collected from inpatients during the period of June 2020 to December 2020. Inpatient samples with initial inconclusive result outcomes, were subjected to repeat testing of the residual sample within 24 – 48 hours.

Results

Of the overall samples tested, 709 (1.7%) yielded inconclusive results. A finding of single gene target detection was observed in 98.4% (n=698) of inpatient samples, with the N gene predominance at 73.6% (n=514). Repeat testing provided negative and positive results in 47% (n=335) and 16% (n=112) respectively, with 28% (n=198) remaining persistently inconclusive. Inconclusive samples which subsequently tested positive detected 2 (56.3%) and 3 (47.3%) gene targets. Inpatients samples testing repeat inconclusive, whilst displaying a single gene target distribution, 66% detected the same gene target at initial and repeat testing (N-N, (n=131); RdRP-RdRP, n=19); with the initial gene target failing to detect the same gene target in 34%, displaying N-RdRP, n=12; N-E, n=6, RdRP-N, n=6; E-RdRP, n=3 and E-N, n=3.

Conclusion

Our relatively low prevalence rate of 1.7% is consistent with other studies. Although clinical history was not furnished, the finding of N gene predominance is explained by inpatients tested at either initial presentation or late stage of COVID-19 infection. Retesting of samples that are initially inconclusive, should be advocated for it allows for establishment of a definitive SARS-CoV-2 outcome.

CONTENTS

Title Page	1
Declaration	2
Dedication	3
Abstract	4
Table of Content.....	5-6
1. Literature Review	10
1.1 SARS-CoV-2 Epidemiology.....	10
1.1.1 SARS-CoV-2 Variant	11
1.1.2 SARS-CoV-2 Transmission.....	12
1.2 SARS-CoV-2 Virology	12
1.2.1 Classification	12
1.2.2 Structure	12
1.2.2.1 SARS-CoV-2 Genome	13
1.3 Clinical Manifestations of SARS-CoV-2	14
1.4 SARS-CoV-2 Laboratory Diagnosis	14
1.4.1 Molecular Assay Test Principles	14-15
1.4.1.1 Molecular Assay Gene Targets	15
1.5 SARS-CoV-2 Ct Values	16
1.6 Result Interpretation	18
1.6.1 SARS-CoV-2 Positive Outcomes	18
1.6.2 Inconclusive SARS-CoV-2 RT-PCR Outcomes	19
1.7 Study Rationale.....	20
1.8 Study Objectives and Aim	21
1.8.1 Study Aim	21
1.8.2 Primary Objectives	21
1.8.2 Secondary Objectives.....	21
2. Materials and Methods	22
2.1 Study Design	22
2.2 Study Setting and Population	22

2.3 Eligibility Criteria	23
2.3.1 Inclusion Criteria	23
2.3.2 Exclusion Criteria	23
2.4 Laboratory Methods	23
2.5 Study Definitions	25
2.6 Data Collection and Management	25
2.7 Statistical Analysis	25
2.7.1 Cost Analysis	25
2.8 Ethical Considerations	26
3. Results	27
3.1 Initial Testing Outcomes	27
3.1.1 Rates of SARS-CoV-2 Positivity.....	28
3.2 Baseline Characteristics of Initial Inconclusive Inpatients.....	28
3.3 Repeat Testing Outcomes	30
3.3.1 Negative Results on Repeat Testing	30
3.3.2 Positive Results on Repeat Testing	31
3.3.3 Repeatedly Inconclusive Results	31
3.4 Overall Gene Target Distribution	31
3.5 SARS-CoV-2 Ct Values	32
4. Discussion	36-42
5. Study Limitations	42
6. Conclusion	42-43
7. References	44-52
8. Appendices.....	53-57

List of Figures

Figure 1.1	Estimated cumulative excess deaths per 100, 000 people during COVID-19 South Africa.....	10
Figure 1.2	COVID-19 cases, hospital admissions and deaths in South Africa from NICD, MRC.....	11
Figure 1.3	Schematic presentation of the structure and genome organization of SARS-CoV-2.....	13
Figure 1.4	SARS-CoV-2 RT-PCR amplification cycle.....	17
Figure 1.5	Estimated variation over time in diagnostic tests for detection of SARS-CoV-2 relative to symptom onset.....	18
Figure 3.1	Flow diagram for the selection of CMJAH Inconclusive inpatient samples.....	27
Figure 3.2	Correlation between the rate of SARS-CoV-2 to inconclusivity and positivity in samples analysed by RT-PCR.....	28

List of Tables

Table 3.1	Demographic and baseline characteristics of inpatients with initially inconclusive outcomes.....	29
Table 3.2	Characteristics of CMJAH Inpatients Repeat Testing Outcomes	30
Table 3.3	Gene Target distribution from initial to Repeat Testing Outcomes.....	32
Table 3.4	SARS-CoV-2 median Ct Values and gene detection in positive and inconclusive inpatients nasopharyngeal swabs.....	33
Table 3.5	Univariate and Multivariate Analysis of factors Associated with positive and negative SARS-CoV-2 Outcomes on Repeat Testing.....	34

Abbreviations

ACE-2	Angiotensin- converting enzyme- 2
CDW	Central Data Warehouse
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
COVID-19	Coronavirus Disease-19
Ct	Cycle threshold
DATCOV	Daily hospital surveillance
HCoV	Human coronavirus
ICTV	International Committee on Taxonomy of Viruses
ICU	Intensive care unit
MERS-COV	Middle East Respiratory Syndrome Coronavirus
MRC	Medical research council
NAAT	Nucleic acid amplification test
NHLS	National health laboratory service
NICD	National institute of communicable diseases
NPS	Nasopharyngeal swab
PHEIC	Public Health Emergency of International Concern
PUI	Person under investigation
RT-PCR	Real time polymerase chain reaction
SARS-COV	Severe acute respiratory syndrome coronavirus
SARS-COV-2	Severe acute respiratory syndrome coronavirus- 2
VTM	Viral transport medium
WHREC	WITS Health and research executive committee
WHO	World Health Organisation

1. Literature Review

1.1 Epidemiology

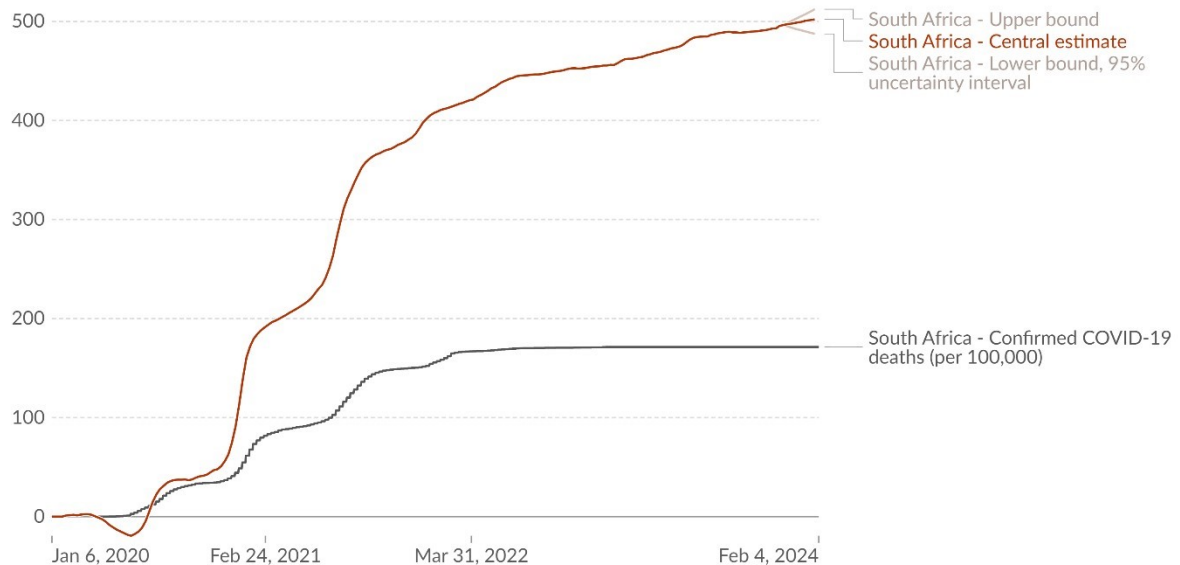
Coronavirus disease (COVID-19) is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a respiratory pathogen reported on the 31st of December 2019 in Wuhan, Hubei, China (World Health Organisation [WHO], 2020). The then novel pathogen was isolated from a cluster of patients presenting with a respiratory illness later identified as COVID-19. The World Health Organisation (WHO) identified COVID-19 as a public health emergency of international concern (PHEIC) and accorded COVID-19 a global pandemic status in January 2020 (WHO, 2020). The devastation of the pandemic is reflected in the high mortality rates with 6.98 million COVID-19 related deaths globally and 102 595 deaths reported in South Africa alone by the end of 2023 (WHO, 2023) (Figure 1.1).

Figure 1.1 Estimated cumulative excess deaths per 100,000 during COVID-19, South Africa

Estimated cumulative excess deaths per 100,000 people during COVID-19, South Africa



For countries that have not reported all-cause mortality data for a given week, an estimate is shown, with uncertainty interval. If reported data is available, that value only is shown. On the map, only the central estimate is shown.



Data source: The Economist (2022); WHO COVID-19 Dashboard

[CC BY](#)

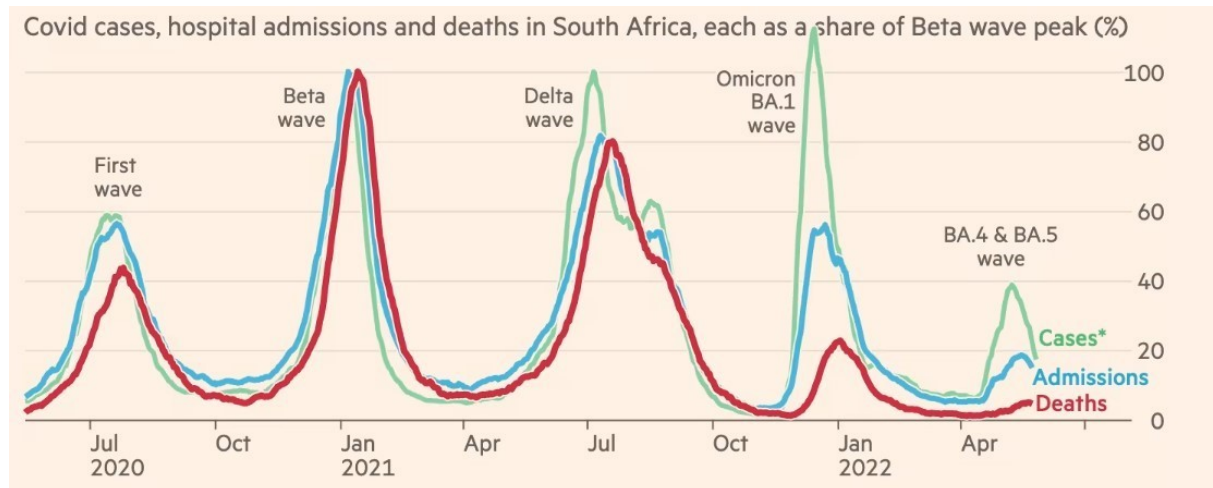
Note: For some countries, all-cause deaths and COVID-19 deaths use different date schemes, in which one refers to when the death occurred and the other to when it was reported. This difference could produce an artificial lag between the two time series.

1.1.1 Variants

Genetic variability of SARS-CoV-2 has led to the formulation of an ongoing surveillance strategy for early detection of potential variants which would otherwise have a negative public health implication. The current WHO variant classification recognises variants of high consequence, variants of concern and variants being monitored (WHO, 2023). The variants have an impact on SARS-CoV-2's transmissibility and severity of clinical symptoms (Cui et al., 2019). In addition, the impact also extends to diagnostic assays which may result in false negative results, should a mutation occur in the part of the gene target for the detection of SARS-CoV-2. During the course of the pandemic, there have been surges and subsequent declines in new cases of COVID-19 and these have been termed "waves". These waves on the whole are caused by the emerging variants of concern (Lin et al., 2022). South Africa has experienced five waves driven sequentially by the ancestral strain with an Asp614Gly mutation, Beta variant

(B.1.351), Delta variant and Omicron BA4 and BA5 subvariants, representing the fourth and fifth wave respectively.

Figure 1.2 COVID- 19 cases, hospital admissions and deaths in South Africa (FT analysis of data from NICD, MRC)



Of interest, from figure 1.2, is the much reduced number of Covid-19 related deaths from the fourth and fifth waves caused by BA4 and BA5 respectively.

1.1.2 Transmission

Infection with SARS-CoV-2 is mainly through contact with respiratory droplets and SARS-CoV-2 infected surfaces. The incubation period varies between 5 to 7 days depending on the circulating variant (Lauer et al., 2020). Although the virus replicates in the upper respiratory tract, viral replication has been reported to occur in the gastrointestinal tract. In addition, the presence of viral RNA has been reported in the peripheral blood (Salzberger et al., 2021).

1.2 Virology

1.2.1 Classification

The International Committee on Taxonomy of Viruses (ICTV) classifies the Severe acute respiratory syndrome-2 (SARS-CoV-2) under the order *Nidovirales* within the

Coronaviridae family. The *Coronaviridae* family is further sub-divided into two subfamilies - *Orthocoronavirinae* and *Torovirinae*, with the *Betacoronavirus* genus encompassing Human coronavirus (HCoV) HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome human coronavirus (SARS-CoV), Middle Eastern respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2. Phylogenetic analysis identified subgenus *Sarbecovirus* as the ancestral origin of SARS-CoV-2 and frequently displays recombination in its evolutionary history (Rastogi et al., 2020).

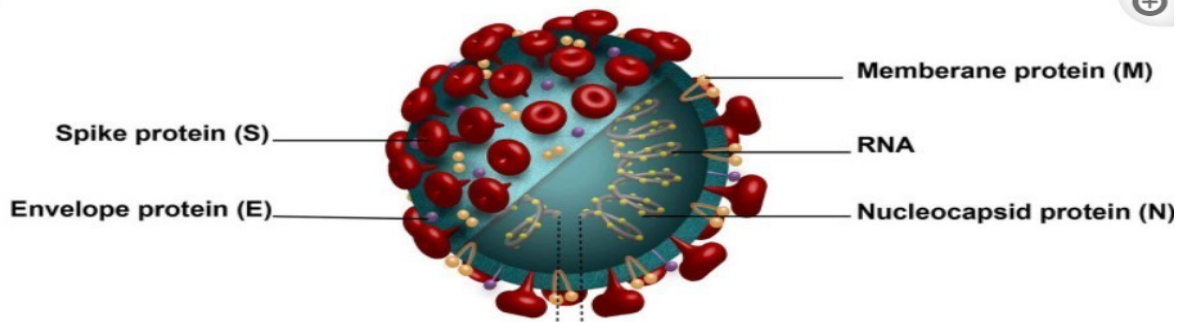
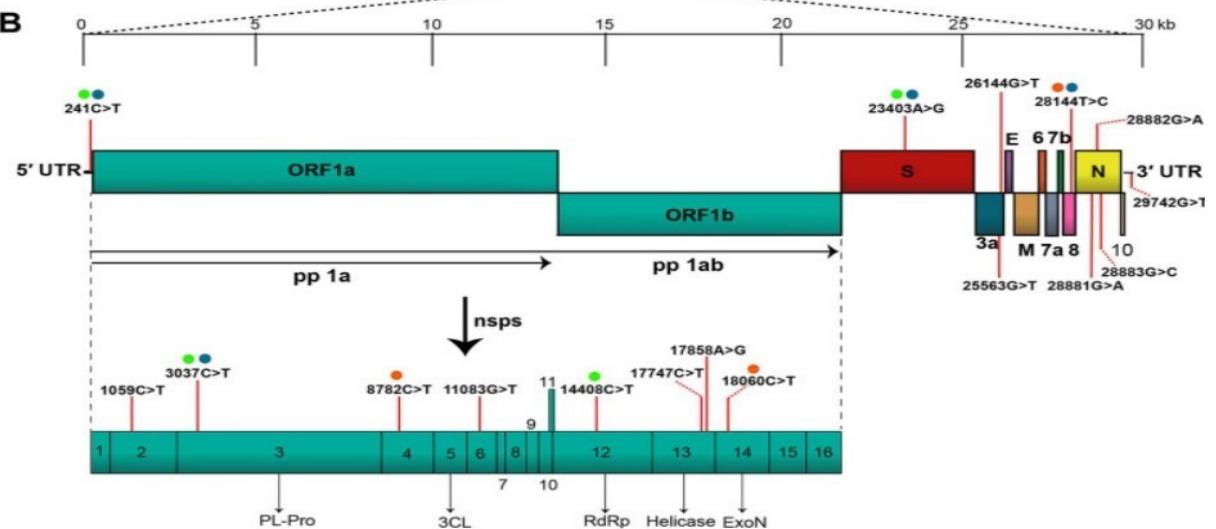
1.2.2 Virus Structure

SARS-CoV-2 is 65 -125 nm in diameter and the 29 to 30 kb single stranded RNA positive sense non-segmented genome is contained inside a capsid (Harcourt et al., 2020; Zhou et al., 2020; Wu et al., 2020). The capsid consist of the nucleocapsid N protein as a ribonucleic complex and the capsid is further surrounded by a membrane that contains three proteins – membrane protein (M) and envelope protein (E) involved in the virus budding process and the spike protein (S). The spike protein binds to angiotensin converting enzyme (ACE-2) receptor, allowing for membrane fusion and ultimate entry of the virus into the cell (Li et al., 2016; Cai et al., 2020).

1.2.2.1 Genome

The SARS CoV-2 genome spans from an untranslated 5'end to a polyadenylated 3'end (Wu et al., 2020), with the 5'end of the genome comprising of two open reading frames (ORF) designated ORF1a and ORF 1b, allowing for the encoding of two polypeptides which are subsequently cleaved to produce 16 non-structural proteins, NSp1-16 (Mousavizadeh et al., 2020). These non-structural proteins play a role in the viral life cycle (Gordon et al., 2020). The 3'-end encodes structural proteins S, N, M and E. The nucleocapsid binds viral genomic RNA in addition to its role in replication and transcription and the viral envelope is in turn composed of the S, M and E proteins (Paules et al., 2020).

Figure 1.3 a and b Schematic presentation of the structure and genome organization of the SARS-CoV-2 (Adapted from Rahimi et al, 2021)

A**B**

1.3 Clinical manifestation

Disease manifestation is dependent on the site where the infection has been established (Mehta et al., 2021). Clinical manifestation of SARS-CoV-2 infection is driven by the presence of ACE-2 receptors in organs such as the heart, intestine, kidney as well as the mucosal lining of the respiratory tract and the vascular endothelial cells. COVID-19 infection is categorised into asymptomatic, mild, moderate to severe form of disease. Symptoms range from a mild febrile illness, dry cough, sore throat to shortness of breath, productive cough with signs of hypoxia. In severe cases, patients present with severe pneumonia, respiratory failure, cardiac failure, multiple organ failure ultimately leading to death (Ganesh et al., 2021).

1.4 Laboratory diagnosis

Nucleic acid amplification tests (NAAT) and serological based assays which amplify viral RNA and viral protein or antibody from respiratory samples, are now widely available. Real time polymerase chain reaction (RT-PCR) is established as “gold standard” method in SARS-CoV-2 diagnostics (Ishige et al., 2024). However, the low sensitivity of RT-PCR assays available at the beginning of the pandemic, were reported to have resulted in a high number of false-negative results (Yang et al., 2020). In that regard, a false negative RT-PCR result could be possibly obtained a few days before symptom onset and at the tail end of SARS-CoV-2 infection - typically from 20 days after symptom onset due to a low SARS-CoV-2 viral load and a viral shedding below analytical RT-PCR sensitivity threshold (Lippi et al., 2020). It is clear however, that the accuracy of tests for SARS-CoV-2 detection in a given sample, will depend on the on the reliability of the SARS-CoV-2 RT-PCR detection methods in use.

1.4.1 Molecular Assay Test Principles

The principle of SARS-CoV-2 RT-PCR is based on the in-vitro amplification of at least two molecular targets (Carter et al., 2020). Commercial SARS CoV-2 assays rely on variable primer probe sets with different gene targets (Carter et al., 2020). The primer probe set selected, will ultimately affect the sensitivity of the assay (Vogels et al., 2020, Dhama et al., 2020). In addition, mutations that may be present on primer probe binding site, could potentially reduce the ability of the assay to detect SARS CoV-2 (Vogels et al., 2020). To mitigate against this, at minimum two different gene targets – with one from the conserved and the other from specific regions of the genome, are recommended to avoid the potential genetic drift and the cross reaction with other endemic coronaviruses and to reduce the chance of spontaneous mutations occurring in both selected gene targets (Tang et al., 2020).

1.4.1.1 Molecular Assay Gene targets

The challenges of decreased sensitivities of various SARS-CoV-2 assays, highlights the importance of strategic selection of gene targets for the detection of SARS-CoV-2. Other than the emergence of mutation in the target area of primer/probe binding, the reasons for reduced RT-PCR sensitivity could be due to the platform itself, the kit and including the methodology in use. Mutations in primer or probe binding sites would not

however be easy to evidence as a reason for a decrease in PCR efficacy (Vogel et al., 2020). The strategy of concomitant detection of two or more gene targets will allow for optimisation of the performance of the SARS-CoV-2 assay.

A number of SARS-CoV-2 molecular targets have been selected for use in various commercial RT-PCR assays. These gene targets, amongst others are nucleocapsid, envelope and the glycoprotein spike protein (Tang et al., 2020; Chan et al., 2020; Konrad et al., 2020; Tombuloglu et al., 2021). Species specific accessory gene targets include RNA dependent RNA polymerase (RdRP), open reading frame 1a (ORF1a) and ORF1b genes (Corman et al., 2020; Lu et al., 2020; Colton et al., 2020). Nucleocapsid gene target and the ORF1a/b, are selected due to their highly conserved nature as well as their unique sequences that are specific for SARS-CoV-2 respectively. E gene on the other hand, serves as a pan-Sarbecovirus marker (Roche et al., 2020).

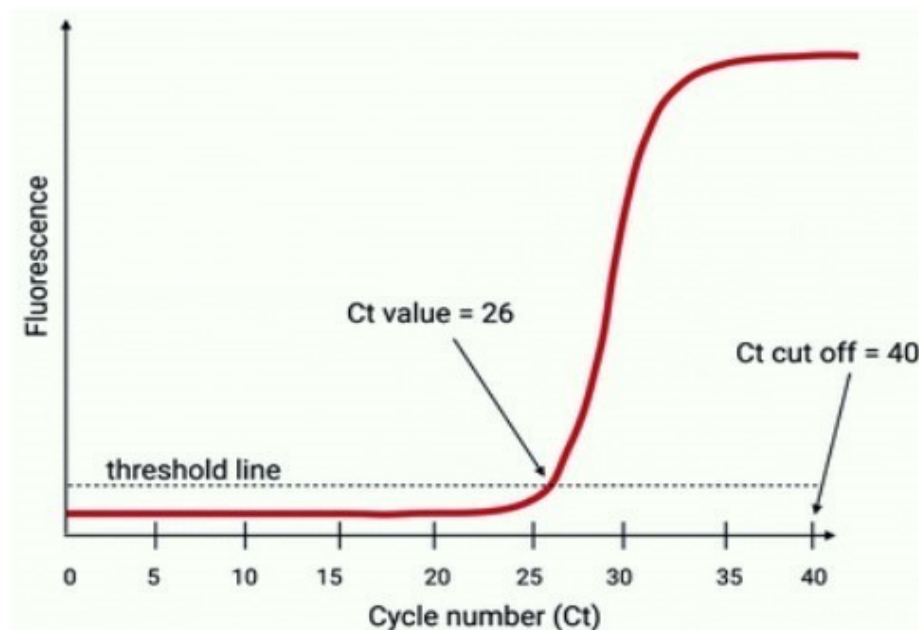
The three WHO recommended SARS-CoV-2 gene targets are RdRP, E and N genes (Corman et al., 2020) with E gene targeted for first line screening and with RdRP and N gene serving as confirmatory targets. The widely utilised Allplex 2019-nCoV (Allplex; Seegene, Seoul, Korea) and TaqPath COVID-19 Combo (ThermoFisher) commercial assays in South Africa are based on the detection of E, RdRP and N gene as well as ORF1ab, N and S respectively.

The group specific E gene target is similar and detectable in all viruses from *Sarbecovirus* subgenus species. RNA dependent RNA polymerase has been shown to have the highest diagnostic sensitivity (Corman et al., 2020; Böger et al., 2021) despite having more than 300 mutations and raises the concern that the mutations could potentially interfere with SARS-CoV-2 detection (Yashvardhini et al., 2021). The N protein and the corresponding mRNA are the most abundant in the replication cycle of coronaviruses (Hiscox et al., 1995; Das et al., 2006). Prolonged SARS-CoV-2 positivity has been widely reported in the literature (D'ardes et al., 2020; Cento et al., 2020; Mallet et al., 2020) as well as positivity before the onset of the clinical symptoms. It has been reported that the N gene was the first and the last to be expressed in the infected cells (Lim et al., 2021; Kim et al., 2021). The basis for species specificity of the RdRP, N and S gene targets is in the fact that these gene targets display low homology when compared to other related coronaviruses (Habibzadeh et al., 2021).

1.5 Ct values

With a maximum of 40 thermal cycles for a typical RT-PCR assay, a cycle threshold (Ct) of a specimen denotes the number of cycles required for the fluorescent signal to cross the threshold (Choudhuri et al., 2020; Engelmann et al., 2021). Viral RNA, measured in Ct value, is detectable as early as the first day of being symptomatic, peaking within 7 days of symptom onset in the majority of COVID-19 infected individuals (Fox-Lewis et al., 2022) (Fig 1.4).

Figure 1.4 SARS-CoV-2 RT-PCR amplification cycle (Adapted from Public Health England, 2020) (Jayakody et al., 2021)



In routine diagnostic practice, RT-PCR Ct values are used as represent the viral load in a given sample. The lower the Ct value the higher the quantity of viral RNA in the sample as an approximate proxy for viral load (Walker et al., 2021).

Interpretation of the patient's SARS-CoV-2 results, is based on the amplification Ct values (Choudhuri et al., 2020; Engelmann et al., 2021). Following review of the performance of quality and internal controls of the assay, together with the gene targets amplified, the test results are then reported as either positive, negative, inconclusive

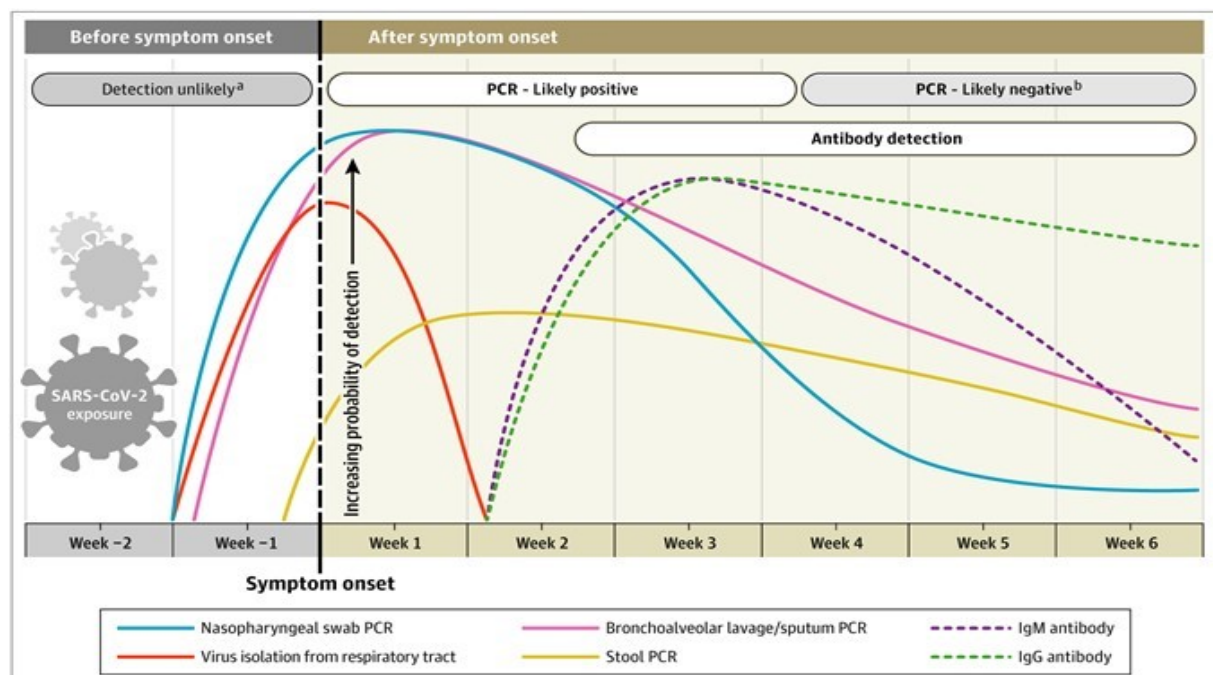
or invalid (Engelmann et al., 2021). It is important to note that Ct values cannot be directly compared between assays of different types due to variation in the sensitivity chemistry of reagents, gene targets, cycle parameters, analytical interpretive methods, sample preparation and extraction techniques (Rhoads et al., 2022).

1.6 Results Interpretation

1.6.1 SARS-CoV-2 Positive Outcome

Generally, and according to most assay manufacturers, a positive sample is one with a Ct value less than 40. Comparison between severely ill patients and those with mild infections reflect differences in viral loads – with the severely ill patients displaying lower Ct values and therefore higher viral loads compared to those with mild COVID19 infections. In addition, when retesting is conducted, PCR positivity in the severely ill group is likely to persist beyond 3 weeks after illness – when a negative result is likely to be an outcome in mildly infected patients on retesting (Yang et al., 2020). A positive PCR in severely ill patients may not necessarily reflect infectivity but rather, possible detection on non-viable viral RNA (Wölfel et al., 2020).

Figure 1.5 Estimated Variation Over Time in Diagnostic Tests for Detection of SARS-CoV-2 Infection Relative to Symptom Onset (Adapted from Sethuraman et al., 2020)



The clinical significance of positive results with high Ct values are challenging to interpret in the absence of clinical history and context.

1.6.2 Inconclusive SARS-CoV-2 RT-PCR Outcomes

Whereas clear-cut positive or negative results are easy to interpret, 3-5% of all samples fall in the grey indeterminate zone, making interpretation a challenge (Bhoyar et al., 2021; Pajudas et al., 2020). Although it is already established that Ct cut offs are in place to ensure specific result outcomes, some SARS-CoV-2 assays have had indeterminate cut-off values introduced. When there is amplification signal of only one of the target genes and the other targets in the assay are not detected, manufacture's recommendation is that the resulting outcomes be called out as inconclusive and the sample merits repeat testing.

The virological causes for these inconclusive outcomes are varied and when E gene target alone is detected, the results signify betacoronavirus infection which is not due to SARS-CoV-2. Different analytical sensitivities of individual viral gene targets, moreso at low viral loads, also have a role to play. In addition, mutations occurring in the target genes, impact the performance of RT-PCR primers or probes (Bhattacharya et al., 2021; Sung et al., 2020; Kim et al., 2020; Peñarrubia et al., 2020).

Other preanalytical and analytical reasons include sample collection and sample quality related issues as well as technical issues related primarily to challenges in RNA extraction (Bhattacharya et al., 2020). It is clear therefore that, retesting of inconclusive RT-PCR results is important as it allows for results confirmation that will ultimately influence decision-making regarding patient management strategy. Inconclusive cases might indicate the initial or late stage of COVID-19 infection which require a different patient management strategy, including isolation and testing approach (Falasca et al., 2020).

The Allplex 2019-nCoV (Allplex; Seegene, Seoul, Korea) and TaqPath COVID-19 Combo (ThermoFisher) commercial assays in use at the CMJAH Virology Laboratory are based on the detection of E, RdRP and N gene as well as ORF1ab, N and S respectively. In our laboratory, a cut off Ct value of ≤ 38 and > 38 is clinically reported as SARS-CoV-2 PCR positive and negative respectively for both platforms. In addition,

both TaqPath COVID-19 Combo Kit and Allplex 2019-nCoV assays, inconclusive outcomes are reported when only one or two of these targets is detected.

It has already been established that inconclusive results in symptomatic patients cause diagnostic, clinical, and infection control uncertainty. These inconclusive samples will most likely require multiple consecutive tests to convert to a negative or positive outcome. Although there are reports of up to 5% SARS-CoV-2- RT- PCR results being inconclusive (Bhattacharya et al., 2020), there is limited information on the extent of results released as inconclusive by diagnostic laboratories, together with the number of repeats and the final outcomes of inconclusive results in the South African public sector setting, where a significant proportion of the SARS-CoV-2 testing is conducted. There is therefore an urgent need to have reliable diagnostic test for SARS-CoV-2 that will provide a clear within turn- around time initial laboratory SARS-Cov-2 testing outcome.

Our goal was to determine the extent of inconclusive results from SARS-CoV-2 testing at CMJAH Virology Laboratory and to discern true and false SARS-CoV-2 outcomes from repeat testing of initially inconclusive results from CMJAH inpatients.

1.7 Study Rationale

Although SARS-CoV-2 RT-PCR is considered the gold standard, interpretation of result outcomes remains a challenge, often calling on the laboratory to retest multiple times. Negative results do not always rule out SARS-CoV-2 infection and are often used in conjunction with other clinical features to determine patient management., The concern of false negatives may be due to sampling error and inappropriate timing of sampling. False negative results may lead to spread of the epidemic in the community. Similarly, the false positive results may lead to unnecessary treatment and mental trauma to the patients. There is therefore an urgent need to have a reliable diagnostic test for SARS-CoV-2 infection. Inconclusive results in symptomatic patients can cause clinical, diagnostic and infection control uncertainty. Inconclusive results require multiple consecutive tests to convert to a negative or positive outcome.

Although up there are reports of up to 5% COVID RT PCR being inconclusive, there is limited information on the extent of results released as inconclusive by diagnostic

laboratories, in the South African public sector setting, where most of the testing is conducted.

Our goal is to determine the extent of inconclusive results from SARS-CoV-2 testing at CMJAH Virology Laboratory and to determine the final outcomes of inconclusive results from inpatients.

1.8 Study Objectives and Aims

1.8.1 Study Aim

This study aims to characterise repeat testing outcomes of inconclusive SARS-CoV-2 RT-PCR results from analytical processing with the Allplex 2019 –nCoV assays in inpatients at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH).

1.8.1.1 Primary objectives

- a) To describe the proportion of CMJAH inpatients with initial inconclusive SARS-CoV-2 RT-PCR results;
- b) To describe the gene target distribution of inpatient's inconclusive testing outcomes.

1.8.1.2 Secondary objectives

- a) To describe gene target patterns in inpatient samples following repeat testing;
- b) To determine factors associated with SARS-CoV-2 RT-PCR repeat testing in CMJAH inpatients;

MATERIALS AND METHODS

2.1 Study Design

The study is a cross-sectional design of SARS-CoV-2 infected inpatients with inconclusive testing outcomes admitted at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and whose SARS-CoV-2 RT PCR was conducted at the National Health Laboratory Service (NHLS) Virology Academic Laboratory.

2.2. Study Setting and Population

The study was conducted at CMJAH, a 1088-bed Johannesburg based hospital with intensive and high-care facilities offering tertiary, secondary and highly specialised services. Diagnostic laboratory work-up is supported by the NHLS. The Virology laboratory is equipped with a number of SARS-CoV-2 platforms as well as Pathologists on site.

The CMJAH Virology Laboratory at the time, was the main centre for SARS-COV-2 testing in Gauteng, receiving samples from inpatients, health care workers (HCWs) and in addition community screening samples. Hospital policy then required that all individuals admitted at the hospital including those with non-COVID-19 related pathologies, to be screened for SARS-CoV-2. As such, the patient population in our study includes symptomatic and non-symptomatic inpatients. CMJAH Area 161, served as main intake and holding area for all incoming patients. Patients would then be allocated to wards once SARS-CoV-2 RT PCR results were released. The following wards were re-purposed as COVID-19 adult inpatient wards – 376, 377, 577, with 587 and 567 serving as Intensive Care Unit (ICU) wards. COVID-19 symptomatic children were admitted to wards 276, 286 and with 357 and 567 serving as paediatric intensive care units. CMJAH staff were managed in Ward 356. In addition, ward 194 was designated for pregnant inpatients with suspected COVID-19. Collected SARS-CoV-2

nasopharyngeal swabs (NPS) were sent to the NHLS Virology laboratory for analytical processing.

The NHLS CMJAH Virology laboratory conducts SARS-CoV-2 RT-PCR through initially use of Allplex 2019-nCoV (Allplex; Seegene, Seoul, Korea) and later with the addition of TaqPath COVID-19 Combo (ThermoFisher). All inconclusive results from SARS-CoV-2 tested samples received from admitted patients from the period of April 2020 to March 2021 and that were subsequently repeat- tested, were requested from the NHLS data repository – Central Data Warehouse (CDW).

2.3. Eligibility Criteria

2.3.1. Inclusion criteria

- All SARS-CoV-2 test results from the inpatient population that were processed on the Allplex 2019-nCoV (Allplex; Seegene, Seoul, Korea) platforms and that initially had inconclusive results and subsequently re-tested on Allplex 2019nCoV;
- Inpatient samples tested between 1st April 2020 to 31 March 2021 for SARSCoV-2 with subsequent repeat testing;
- Inpatient samples of all ages;
- Inconclusive samples repeat-tested within 24-48 hours.

2.3.2 Exclusion criteria

- All inconclusive samples from community screens;
- All inconclusive samples that were not subsequently re-tested;
- Initially inconclusive results repeated on newly recollected samples;
- Inconclusive samples repeated > 48 hours from initial testing; □ All results with missing patient laboratory and clinical data.

2.4 Laboratory Methods

The NHLS Virology Laboratory accepted nasopharyngeal swab samples transported in viral transport medium (VTM) or phosphate buffered saline (PBS) from people under investigation (PUIs) for SARS-COV-2 diagnostic testing. The samples were extracted

in the Seegene Nimbus or the Magnipure extraction platforms, followed by real time amplification on the thermocycler.

For amplification, the Allplex RT-PCR assay which amplifies 3 specific targets has the E gene serving as a screening test (pan-sarbecovirus target), and the RdRP and N genes which are SARS-CoV-2 species specific, serving as a confirmatory test. For amplification, the protocol followed as per manufacturer instructions, involves a master mix preparation of 5µl one-step buffer, 5µl water, 5µl 2019 nCoV MOM and real-time 2µl enzyme as well as 8 µl of quality controls. A total reaction mixture of 25µl was aliquoted into the amplification plate. In addition, PCR inhibitors were monitored by the inclusion of an internal control and the reaction mixture amplified in the Seegene Allplex platform. Results from the Allplex thermocycler were analysed and uploaded by the software for pathologist decision and resulting.

Worksheets with run information for all the specimens with inconclusive results were checked for validity of specific runs. In addition, the amplification curves were visually checked for atypical amplification curves above the threshold that signal inconclusive outcomes. However, checking if inconclusive specimens were not adjacent to another highly positive specimen (defined as Ct <20) was not conducted – hence cross contamination could be ruled out.

According to the laboratory standard operating procedure, inconclusive samples were repeat-tested. For the retesting was conducted by re-extracting nucleic acids from the residual samples. We selected only samples that were initially tested on Allplex SARSCoV-2 assay (Seegene Inc.; Seoul, Korea) and rested on the same platform. According to the standard operating procedure (SOP), retesting would be conducted within 2448 hours of an inconclusive result outcome.

2.5 Study Definitions

Our laboratory defined criteria for result interpretation are as follows: SARS-CoV-2 RT PCR is reported as positive when all 3 gene targets are detected. Results are invalid when the internal control has not amplified. Inconclusive results on the other hand are called out when only 1 or 2 gene targets are detected and the Ct values is less than 38.

2.6. Data Collection and Management

Variables from SARS-CoV-2 test results, that met the inclusion criteria were requested from CDW. These included episode number, date of birth, gender, ward, dates of initial and repeat testing and gene targets detected – all captured on Trakcare laboratory Information System. For the following clinical variables, approval was sought and attained from DATCOV sentinel surveillance system: i) Demographic, ii) anthropometric measures and iii) comorbidities - in particular HIV and CD4 T cell count. SARS-CoV-2 RT-PCR results from April to May 2020 were not available. The study period therefore included data from June to December 2020, allowing for only a 7 month's data analysis. In addition, data from January 2021 to March 2021 was missing and therefore not extracted.

2.7 Statistical Analysis

Descriptive statistics were determined for continuous measures using the median and interquartile ranges (IQR), whereas categorical data was presented as frequencies and percentages. Where appropriate, Chi-square, paired t-test, Pearson's correlation coefficient and the Wilcoxon signed-rank test were used to evaluate statistical differences. Univariate and multivariate logistic regression analysis was used to examine the relationship of independent variables with the primary outcomes. All variables were included in the multivariate analysis model with logistic regression using odds ratio. Variables with *p values* < 0.05 were be considered statistically significant. All data was analysed using Microsoft Excel and Graph Pad Software (Graph Pad Inc., USA).

2.7.1. Cost Analysis

The study aimed to reflect major and minor laboratory costs associated with repeat testing of inconclusive SARS-CoV-2 samples during the pandemic. Costing of consumables such as sampling swabs, viral transport media and test kit reagents were going to be considered and calculated per test. Microsoft excel spreadsheet as well as the Stata statistical package was going to be used to capture both the parameters of test activity as well as the breakdown of test kit contribution. A bottom up method to

estimate cost per result was planned for as well as soliciting quotations will be solicited from the manufacturer as well as from the National Health Laboratory Service (NHLS). However, due to academic time constraints the cost sub-analysis of this project will be conducted separately from the dissertation and published.

2.8. Ethical Considerations

The study protocol was submitted to the University of Witwatersrand Health Research Ethics Committee and approval granted (Appendix i). In addition, approval was also sought from the hospital management of CMJAH as well as from the NHLS CDW and DATCOV (Appendices ii, iii, iv) to allow for access to patient SARS-CoV-2 laboratory results and clinical information respectively as well as information on comorbidities.

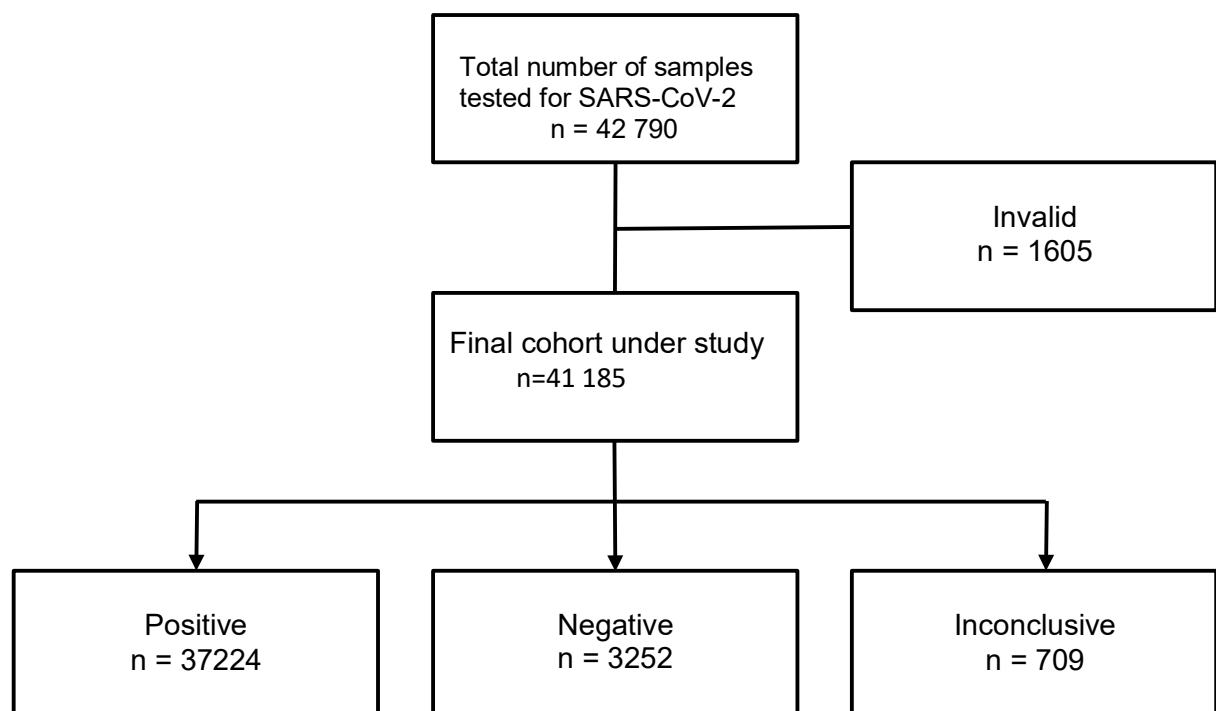
3. RESULTS

3.1 Initial Testing Outcomes

In our laboratory, during the period from June to December 2020, 42 790 nasopharyngeal swabs (NPS) were collected from inpatients at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and tested for SARS-CoV-2 using the Allplex SARS-CoV-2 assay.

Of 42 790 samples analysed, 1605 (3.7%) were invalid and subsequently excluded from the study and of the remaining overall 41 185, 37 224 (90.4%) and 3252 (7.9%) samples tested negative and positive for SARS-CoV-2 respectively. Seven hundred and nine SARS-CoV-2 inconclusive samples where either E, RdRP or N was isolated regardless of the Ct value, were identified.

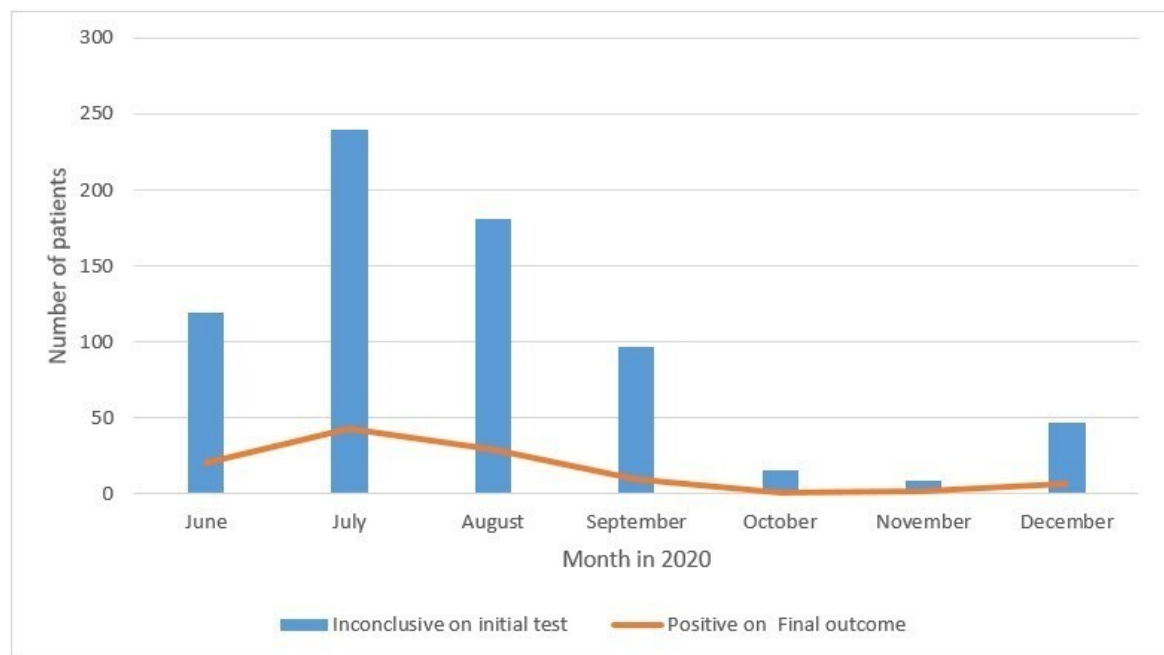
Fig. 3.1 Flow diagram for the selection of CMJAH inconclusive inpatient samples



3.1.1 Rates of SARS-CoV-2 Positivity

Our study period coincided with the first pandemic wave characterised by the circulating WT D614G variant, with the peak in SARS-CoV-2 positivity occurring in July 2020. From stratifying both positive and inconclusive samples by month covering the study period, we observed that as the number of samples testing SARS-CoV-2 positive in our laboratory increased, the number of inconclusives also displayed a corresponding increase (Fig 3.2).

Figure 3.2 Correlation between the rate of SARS-CoV-2 inconclusivity and positivity in samples analysed by RT-PCR



3.2 Baseline characteristics of Initial Inconclusive Cohort

Of the 709 inpatients with inconclusive results, the median age (interquartile range [IQR]) was 34 years [25.5-49.5 years; $p < 0.810$], while 445 (63 %) of the patients were female. The cohort was predominately adult inpatients ($n=643$; 91%), with only 66

(9.3%) children. Although the clinical background on tested inpatients was not provided for, requests for testing from laboratory requisition forms were determined to be predominantly (n=378; 53.3%) from the medical wards (Table 1).

Table 3.1 Demographic and baseline characteristics of inpatients with initially inconclusive outcomes.

Characteristic	Overall (N=709)	N (%)
Age, median (IQR)	34 (25.5, 49.5)	
Adults	643.0	90.7
Children (<14yrs)	66.0	9.3
Gender, n (%)		
Female	445.0	62.7
Male	264.0	37.2
Sub-specialities		
Medical	327.0	52.3
O and G	143.0	22.8
Surgical	130.0	20.8
Oncology	20.0	3.2
Transplant	5.0	0.8
Paediatrics		
Medical	51.0	60.7
Oncology	12.0	14.3
Surgical	7.0	8.3
Transplant	0.0	0.0
Others	14.0	16.7

Of the 709 inconclusive results, the majority (n=698; 98.4%) of Allplex based gene targets detected, were single genes – N, RdRP or E. The remaining 11 had 2 genes detected in the following combinations – RdRP+/N+ (n=7; 0.4%), E+/N+ (n=3; 1%) and E+/RdRP+ (n=1; 0.1%). The N gene target was the more frequently detected, with 73.6% (514/698), 13.3% (93/698) and 13.0% (91/698) being N, RdRP and E gene respectively.

The median Ct values of N, RdRP and E were 38, 37 and 36 respectively. The Ct range of N (minimum = 29.0, maximum = 39.0) was much narrower than that of E (minimum

= 26.0, maximum = 39.0) and RdRP (minimum =24,0 maximum 39,0). The CMJAH Virology defined Ct cut off for positive detection of 38 was applied to filter out all gene targets with Ct values > 38. The revised frequency of detection was therefore 484 (69.3%), 81(11.6%) and 86 (12.3%) of N, RdRP and E respectively – with the N gene consistently the most frequently detected.

3.3 Repeat Testing Outcomes

The Laboratory's SOP stipulate that SARS-CoV-2 specimens yielding an initial inconclusive outcome, should be repeat-tested within 24 hours. Our finding was that the time interval between all original specimen samples testing inconclusive and repeat testing being conducted, was 12 to 48 hours. From the inconclusive cohort of 709, the time interval was 1.2 ± 1.2 , 1 ± 1.3 and 1.1 ± 1.4 ($p < 0.000$) for samples that yielded a positive, a negative and a persistent inconclusive SARS-CoV-2 outcome on repeat testing (Table 3.2).

3.3.1 Negative Results on Repeat-Testing

For this analysis, of the 709 initially inconclusive samples, 47.2% (n=335) on repeat testing were negative, thereby pointing to the likelihood that the initial test was most likely false-positive.

Table 3.2 Characteristics of CMJAH Inpatient Repeat Testing Outcomes

Variable	Total (n=709)	Positive (n=112; 16%)	Negative (n=335; 47.2%)	Inconclusive (n=198; 28%)	Invalid (n=64; 9.0%)	p-value
Age, median (IQR)	34 (25.5, 49.5)	34 (27.3, 47.8)	34 (25, 47)	35.5 (26, 51.3)	37 (27, 50.0)	0.582
Adults	643 (90.7)	104 (92.9)	301 (89.9)	181 (91.4)	57 (89.1)	0.747
Children (<14yrs)	66 (9.3)	8 (7.1)	34 (10.1)	17 (8.6)	7 (10.9)	
Gender, n (%)						
Female	445 (62.8)	80 (71.4)	201 (60.0)	125 (63.1)	39 (60.9)	0.187
Male	264 (37.2)	32 (28.6)	134 (40.0)	73 (36.9)	25 (39.1)	
Hospital ward						
Medical	327 (46.1)	47 (41.9)	154 (46.0)	97 (48.98)	29 (8.9)	0.693
O and G	143 (20.2)	30 (26.8.)	63 (18.8.)	37 (26)	13 (9.1)	0.295
Surgical	130 (18.3)	19 (17.0)	68 (20.3)	33 (16.7)	10 (15.6)	0.640

Oncology	20 (2.8)	4 (3.6)	8 (2.4)	7 (3.5)	1 (1.6)	0.754
Transplant	5 (0.7)	0 (0)	1 (0.3)	2 (1)	2 (3.1)	0.066
Paediatrics						
Medical	51 (7.2)	6 (5.3)	25 (7.4)	14 (7.1)	6 (9.4)	0.786
Oncology	12 (1.7)	2 (1.8)	6 (1.8)	2 (1)	2 (3.1)	0.713
Surgical	7 (1)	2 (1.8)	4 (1.2)	1 (0.5)	0 (0)	0.575
Transplant	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	-
Others	14 (2)	2 (1.8)	6 (1.8)	5 (2.5)	1 (1.6)	0.931
Ct-Values	36.9 ± 2	36.1 ± 2.4		36.9 ± 1.9	36.6 ± 2.1	0.000
Time interval (days)		1.2 ± 1.2	1 ± 1.3	1.1 ± 1.4	1.9 ± 2.1	0.000

3.3.2 Positive Results on Repeat Testing

Only 112 out of 709 (16%) inpatients with an initial inconclusive test result, tested positive in the follow-up test on the same sample. The time interval from an initial inconclusive to positive follow up results was 1.2 ± 1.2 days. A change in Ct value from an initial inconclusive to the final positive outcome was observed – with the Ct-value decreasing from an initial inconclusive median Ct [IQR] result of 38 [37–38] to a positive median Ct [IQR] result of 34 [32–36].

3.3.3 Repeatedly Inconclusive Results

Of significance were 198 (27.9%) inpatient samples that were repeatedly inconclusive. As per protocol of the CMJAH Virology laboratory, inpatients with repeat inconclusive results lead to a request for a new nasopharyngeal swab on the inpatients concerned.

3.4 Overall Gene Target Distribution

With 698 (98.4%) initial inconclusive outcomes being single gene targets, the most frequently detected single target was the N gene at 73.6% in CMJAH inpatients. From repeat testing, where the initial outcome was repeatedly inconclusive, the predominance of N (66%) persisted (Table 3.3).

Where repeat testing yielded a positive outcome (n=112), the presence of two and three gene targets was observed in 52.6% and 47.3% respectively. The two gene target combination of RdRP+/N+ and N+/E+ predominated at a frequency of 27 (24.1%) and 26 (23.2%) respectively.

The remaining 53 (47.3%) had all 3 gene targets detected, with 34 (67%) with a Ct value <36.

Table 3.3 Gene target distribution from initial to repeat testing outcomes

Targeted gene	Initial inconclusive outcome (n=709)	N%	Repeat testing Positive Outcome (n=112)	N%	Repeat Testing Inconclusive Outcome (n=198; N%)	N%
Single Gene Targets						
RdRP gene only (%)	93	13.3	0.0	0.0	47.0	23.7
N gene only (%)	514	73.6	0.0	0.0	132.0	66.7
E gene only (%)	91	13.0	0.0	8.0	18.0	9.1
2Gene Targets						
E gene and N gene (%)	3.0	0.4	26	23.2	0.0	0.0
RdRP gene and N gene (%)	7.0	1.0	27	24.1	1.0	0.5
E gene and RdRP	1.0	0.1	6.0	5.3	0.0	0.0
3Gene Targets						
E, RdRP, N	0.0	0.0	53.0	47.3	0.0	0.0

3.5 SARS-CoV-2 Ct values

The Ct values of 36.9 ± 2.4 for initially inconclusive, 36.1 ± 2.4 positive on repeat testing and 36.9 ± 1.9 for repeated inconclusive were significantly different between the three groups ($p > 0.000$). The overall average Ct value of initial inconclusive (36.9 ± 2) results reflect high Ct values that are close to the laboratory defined cut off Ct value. The median Ct values in repeat-tested positive cases with the E+/N+ pattern was 37, RdRP+/N+ with a median Ct of 35 and E+/RdRP+ with a median Ct 36 as shown in Table 3.4.

Table 3.4 SARS-CoV-2 median Ct values and genes detected in positive and inconclusive swabs

	Positive (N = 112)		Inconclusive (N = 198)	
Number of genes detected	N%	Median CT* (IQR) [range]	N%	Median CT* (IQR) [range]
1	0.0 (0.0%)	-	198.0 (100)	37 (36-38) [25-39.78]
2	59 (52.6%)	36.5 (35-38) [20 - 39]	0.0	-
3	53 (47.3%)	34 (32-36) [15- 39]	0.0	-
Genes detected				
N only	0.0	-	133.0	38 (37-38) [25-39.78]
E only	0.0	-	18.0	36 (35-36) [33-38]
RdRP only	0.0	-	47.0	36.87 (35-37) [29-39]
E and N	26	37 (35-38) [20-39]	0.0	-
RdRP and N	27	35 (30-39) [30-39]	0.0	-
E and RdRP	6	36 (35-36) [30-36]	0.0	-

Table 3.5 Univariate and multivariate analyses of factors associated with positive and negative SARS-Cov-2 outcomes following repeat testing of an initial inconclusive result in inpatients at CMJAH: June to December 2020

Variable	Positive		Negative		<i>p</i>	Univariate			Multivariate		
	n	%	n	%		OR	95% CI		OR	95% CI	
							Lower	Upper		Lower	Upper
Adults	104	92.9	301	89.9	0.454	1.468	0.659	3.274	5.583	.258	120.906
Children (<14yrs)	8	7.1	34	10.1	0.454	0.681	0.305	1.518			
Gender, n (%)											
Female	80	71.4	201	60	.032	1.667	1.047	2.652	1.576	.879	2.827
Male	32	28.6	134	40	.032	0.600	0.377	0.955			
Testing interval ≤ 24 hours	25	22.3	99	29.6	0.146	.685	.414	1.133	.680	.389	1.190
Hospital ward											
Medical	47	42	154	46	0.511	0.850	0.551	1.310	1.361	.155	11.942
O and G	30	26.8	63	18.8	0.081	1.580	0.958	2.604	2.499	.280	22.313
Surgical	19	17	68	20.3	0.492	0.802	0.458	1.405	1.373	.149	12.684
Oncology	4	3.6	8	2.4	0.506	1.514	0.447	5.127	2.449	.201	29.901
Transplant	0	0	1	0.3	1.000	-	-	-			
Paediatrics											
Medical	6	5.4	25	7.5	0.525	0.702	0.280	1.758	4.941	.122	200.017
Oncology	2	1.8	6	1.8	1.000	0.997	0.198	5.012	9.281	.228	378.241
Surgical	2	1.8	4	1.2	0.643	1.505	0.272	8.327	11.954	.187	763.919
Others	2	1.8	6	1.8	1.000	0.997	0.198	5.012			
E-1 gene	13	11.6	52	15.5	0.355	0.715	0.373	1.368	14.498	1.644	127.878
RdRp-1 gene	24	21.4	41	12.2	0.020	1.956	1.120	3.414	52.380	5.840	469.798
N-1 gene	83	74.1	243	72.5	0.807	1.084	0.666	1.762	47.171	5.263	422.767
Ct-Values (Mean ± SD)	36.1 ± 2.4		37.3 ± 1.7		0.000				.716	.632	.811
Age, median (IQR)	34 (27.3, 47.8)		34 (25, 47)		0.689				1.004	.987	1.021

Factors found to be associated with SARS-CoV-2 positivity from repeat tested samples were likely to be adults 92.9% (OR: 1.46, 95%CI:0.65-3.27), being female 71.4% (OR: 1.66, 95%CI: 1.047- 2.65) and for adults from O&G and oncology wards 26.8% (OR: 1.580 95%CI:0.958-2.604; 3.6% (OR:1.514 95%CI:0.447-5.177) respectively. In paediatric patients, admission to the surgical ward, was associated with SARS-CoV-2 positivity on repeat testing - 1.2% (OR:1.505, 95%CI:0.272-38.327).

4. DISCUSSION

There is no test that gives a 100% accurate result and as such tests need to be evaluated to determine their sensitivity and specificity ideally by comparison with a gold standard. Since the onset of the pandemic, the SARS-CoV-2 real-time RT-PCR not only has been regarded as the gold standard but continues to be widely used for detection of infection. Throughout the pandemic to date, SARS-CoV-2 RT-PCR negative and positive outcomes presented little or no challenge in the clinical environment. Inconclusive results however create a diagnostic uncertainty and the challenge of inconclusive results continues to be reported. In this present study we therefore aimed to determine the extent of true or false positive findings from initially inconclusive results from SARS-CoV-2 testing at a large academic hospital in Johannesburg South Africa.

Inconclusive SARS-CoV-2 RT-PCR results reduce diagnostic confidence in terms of establishing a definitive COVID-19 diagnosis, direction in terms of clinical management as well as infection control preventative measures that can be undertaken (Chan et al., 2020). In addition, inconclusive results released by the diagnostic laboratory, are not always easy for the clinicians to discern (Yang et al., 2021). It is for these reasons that repeat testing following a finding of an inconclusive result is advocated for as this diagnostic laboratory practice allows for a definite conclusion of the test result in most cases (Boeckmans et al., 2021; Munne et al., 2021). The standard practice following an inconclusive result outcome in our laboratory, is to retest the same nasopharyngeal residual sample that yielded an initial SARS-CoV-2 inconclusive result within the laboratory defined interval of 24 - 48hrs. In our study, the time to repeat testing was within the stipulated time limit and does not represent variation factor in the final outcomes.

Charlotte Maxeke Johannesburg Academic Hospital during our study period, admitted patients categorised as having moderate to severe COVID-19 symptomatic infection. In addition, the screening algorithm in place at CMJAH mandated SARS-CoV-2 testing of all hospital attendees including asymptomatic individuals coming in as

preadmissions for planned procedures. We were not able to however, confirm a history of

COVID-19 related symptoms nor its absence in inpatients admitted for elective procedures, clinical follow-up, obstetrics and gynaecology, those presenting at casualty and health care workers. However, as there were COVID-19 and non-COVID19 designated wards, in this study wards are used as a proxy for stratifying inpatients into symptomatic and asymptomatic categories for COVID-19.

A relatively low rate of 4 – 5% SARS-CoV-2 inconclusive results has been generally reported (Bhoyer et al., 2021; Pujudas et al., 2020). In our study, inconclusive results conducted on the Allplex 2019-nCoV (Allplex; Seegene, Seoul, Korea) platform were detected in 709 (1.7%) of the overall 41185 inpatient samples tested for SARS-CoV-2 during the first COVID-19 wave driven by the ancestral strain. The 1.7% prevalence rate of SARS-CoV-2 inconclusivity coincides with the previous findings of 0.3% to 2.9 % from studies conducted in the United States, Belgium, Bulgaria, Ireland, Indonesia and Korea (Dolan et al., 2024; Boeckmans et al., 2021; Stoykova et al., 2022; Yang et al., 2021; Ardianto et al., 2021; Lim et al., 2021). Jeon et al reported much higher inconclusive rates of 81.7% in their study reflecting the dynamic factors influencing the rate of inconclusive results in different study settings (Jeon et al., 2023). Of note is that studies supporting our inconclusive findings were also conducted during the first pandemic wave, however the fact that different testing platforms with different gene targets and different patient sample sub-populations were used, makes a comparison of these SARS-CoV-2 results a challenge. In addition, we observed a correlation between the frequency of SARS-CoV-2 positives detected monthly and the number of corresponding inconclusive results by month. However, the higher the total number of samples tested, the higher the inconclusive and positive detection rates - wherein, as the number of the inpatient samples testing positive increased, the number of inconclusive inpatient samples also increased.

In our study, the majority of initially inconclusive results were characterised by a high frequency of single gene target positivity (n=698; 98.4%). Overall, the N gene was solely amplified in 74% (n=514) of samples across the study inpatient subpopulation, with no preponderance for gender and age. The predominance of the isolated N gene in inconclusive results is supported by studies reporting frequency of N gene detection at 65% and 100% respectively (Ardianto et al., 2022; Stoykova et al., 2022). The finding

of a high frequency of the isolated N gene target compared to the E and RdRP gene targets, can be attributed to the stability of the N gene, a gene that is conserved, possessing less nucleotide variation compared to the E gene and RdRP gene (Grifoni et al., 2020). The N protein and its corresponding mRNAs dominate the replication cycle of coronaviruses and the partial amplification of viral RNA remnants by SARS-CoV-2 RT PCR, together forms the basis of the rationale that inconclusive single gene N target outcomes in inpatient samples represents initial or late stage of COVID-19 infection (Das et al., 2006; Falasca et al. 2020; Kim et al., 2020; Lim et al., 2021). Of the 514 inpatient samples with inconclusive single N gene positivity, 190 (36.9%) were inpatients from the designated medical COVID-19 wards and thus assumed to be COVID-19 symptomatic. The remaining 324 (63.0%) inpatients were SARS-CoV-2 screening cases assumed to be asymptomatic. There are also reports of a high proportion of inconclusive SARS-CoV-2 RT PCR results being false positive, especially amongst asymptomatic patients. In a setting of low prevalence, even a test with high specificity could generate a substantial percentage of false positive results (Yang et al., 2020; CDC 2020). In our study therefore, the hospital policy of screening all patients with low pre-test probability for COVID-19 infection- including asymptomatic preadmission and presurgical attendees, we assume inevitably led to false positive results. Since clinical decisions including timing of elective surgery may be based in part on COVID-19 screening test results, false-positive results can delay care for patients and have unintended consequences, including delayed surgery or delays in diagnosis, as has been reported in the literature (Schizas et al., 2020; Katz et al., 2020). It is therefore important for clinicians to be able to understand the meaning of inconclusive tests.

There are a number of factors that may cause inconclusive results ranging from inadequate nasopharyngeal sampling, cold chain transport temperature deviations, poor quality of viral transport medium (VTM) and extraction failure (Bhattacharya et al., 2020). In addition, false positivity could be found in the non-specific binding during the late phases of the PCR reaction –an assumption that is supported by the relatively high Ct values obtained (Bhattacharya et al., et al, 2020).

Re-testing in case of inconclusive results has been widely advocated for since repeat testing allows for a definite conclusion of the test result in most cases (Boeckmans et al., 2021). The retesting of the 709 inconclusive inpatient samples in our study cleared

112 samples (16%) as positive, 335 samples (47.5%) as negative and 198 samples (28%) as inconclusive.

The probability of a negative outcome from an initial inconclusive test, has also been recognised in a number of studies (Gubbay et al., 2021; Lim et al., 2021). Our study demonstrated that 47.2% of the SARS-CoV-2 inconclusive RT-PCR results were subsequent negatives on retesting – this indicating that the index test was most likely false positive, although it is still possible that SARS-CoV-2 RT PCR partially amplified small amounts of remnant RNA in the initial testing (Boeckmans et al., 2021; Munne et al., 2021). The majority of these inpatient samples were from the medical, obstetrics and surgical wards at 36.7%, 21.5% and 20% respectively. Our finding of subsequent negative results is consistent with reports of 31.5%, 42.1%, 46%, 52% from studies conducted in South Korea and the United States respectively (So et al., 2020; Jeon et al., 2023; Green et al., 2020; Yang et al., 2021). Higher false positive of 86% and 86.9% results were reported in studies in the Netherlands and Belgium (Rondaan et al., 2023; Boeckmans et al., 2021).

In addition, a negative result when following retesting of an inpatient inconclusive sample implies that there might have been an error in the previous inconclusive result. These results suggest that contamination from adjacent wells with positive samples or previously analysed positive samples, cannot be ruled out. Human errors from mishandling of samples or reagent instability are factors that should also be considered (Hong et al., 2020; Sung et al., 2020; Kim et al., 2020).

Given a high analytical sensitivity of SARS-CoV-2 RT PCR of over 99%, repeat-tested inpatients that yield a positive outcome can be considered true positives. The finding of 16% subsequent SARS-CoV-2 RT PCR positive inpatient samples from repeat testing in our study, can thus be regarded as true positive (Zhou et al., 2020; Pfeifferle et al., 2020; Smithgall et al., 2020; Cordes et al., 2020; Zhen et al., 2020).

The majority of these inpatients (n=71; 63.4%) were from non-COVID-19 designed wards and therefore assumed to be COVID-19 asymptomatic. Of the 112 subsequently positive inpatients, 61 (54.5%) and 51 (45.5%) were positive in only 2 or 3 gene targets. providing assurance in overall results.

Whilst Ct values are not directly comparable between studies, according to the Public Health England study, only samples with Ct value <37 were positive on repeat testing of the same nasopharyngeal swab (Ladhani et al., 2020; Riley et al., 2020). Of 112

CMJAH inpatients with a repeat-testing positive outcome, 34 (67%) had all 3 target genes detected in addition to having a Ct value < 36 – thus likely reflecting true positivity of these samples. The remaining 59 inpatients had 2 gene target detected with a combination RdRP+/N+ (n=27), N+/E+ (n=26) and E+/RdRP+ (n=6). Our results are supported by findings from Ardianto et al showing that when two genes (N+/E+) were detected, the probability of positive result on retest was significantly higher (80%) (p-value = 0.001) than when only one gene was detected at 45.16% (Ardianto et al., 2022).

One hundred and ninety-eight (28%) of samples in our initially inconclusive inpatient cohort, returned inconclusive outcomes following repeat testing. Individuals with repeated inconclusive results, without a confirmed history of COVID-19 as is the case with our cohort - are often reported to be patients in the incubation period or at an early stage of infection (Sung et al., 2020).

Although two thirds (n=131; 66%) of repeated initially inconclusive samples displayed identical gene target amplification at retesting, 34% (n=67) were persistently inconclusive and had a different single gene target detected. The majority (66%) of all inconclusive results we obtained, amplified the N gene at initial and repeat testing (N+N+). This is an expectation as the N gene is reported to be the first and last to be expressed in the infected cells (Lim et al., 2021; Kim et al., 2020). The other initial-repeat testing inconclusive result combinations were N+ - RdRP+ (n=12; 25%), RdRP+ - RdRP+ (n=19; 9.6 %), N+ - E+ (n=6; 3%), RdRP+ - N+ (n=6, 3%) E+ - RdRP+ (n=3; 1.5%) and E+ - N+ (n=3; 1.5%). These changes in gene target detection from initial to repeat testing may indicate that the same genes targets may have been present in the sample or that some gene targets are lower than the analytical sensitivity of the assay used (Drew et al., 2020; Lim et al., 2021). In addition, the different analytical sensitivity of the RT-PCR assay for the individual genes could also play a role, with each primer probe set having a specific target and even single-point mutations that potentially could lead to amplification failure of the corresponding gene (Artesi et al., 2020; Miller et al., 2021). Gene target combination of initial inconclusive E gene target changing to a different gene target on repeat testing, could be a reflection of the non-specificity problems of the gene as well as E gene's vulnerability to sample contamination (Benrahma et al., 2020; Lim et al., 2021; Bezier et al., 2020). Overall, our data indicated

that retesting inconclusive samples was beneficial, particularly when two genes were detected, but was ineffective when only E gene was detected.

In laboratory medicine, it is widely recognised that restricting diagnostic test results to either a positive or negative outcome, whilst desirable for diagnostic decision making, is not a reality in a clinical setting as there often is a subset of results that are relatively uninformative, ultimately categorised as inconclusive or indeterminate (Feinstein et al., 1990). Our study supports the finding that when the SARS-CoV-2 positive rate is high, there likely will be a corresponding increase in the inconclusive rate ultimately increasing the amount of resource input through warranting repeat testing until a definitive diagnosis is made. To reduce the consumption of resources, an inconclusive test should be interpreted flexibly with the patient's characteristics or even the epidemiological setting at the time of repeat testing (Stoykova et al., 2022). Therefore, there is a high probability of an initially inconclusive result yielding a positive outcome on repeat testing when the patient is local and the positivity rate at the time is high.

Three hundred and thirty-five (47.2%) of our initially inconclusive samples tested negative following repeat testing – making subsequent negative outcomes the most frequent finding on repeat testing. The initial inconclusive signal in those that are newly infected with COVID-19 or those that are asymptomatic but with past COVID-19 infection, necessitate retesting – allowing for a definitive COVID-19 diagnosis.

With association between Ct value and SARS-CoV-2 viral load, inconclusive results from COVID-19 patients may indicate that the viral load decreased during recovery from infection and as such inconclusive results could derive from a natural process during follow-up of patients with positive SARS-CoV-2 RT-PCR. Furthermore, the SARS-CoV-2 diagnostic RT-PCR assays detect viral RNA even when the virus has lost its infectivity (Atkins et al., 2020; Dighe et al., 2020) therefore, inconclusive results associated with a lower viral load than positive results may indicate no infectivity. In addition, there is a school of thought that mutations of SARS-CoV-2 target genes have a bearing of inconclusive results (Penarrubia et al., 2020). However, the concern is challenged by the finding of alternating target gene targets upon request i.e. initial detection of N gene and replaced by RdRP detection on retesting without the presence of N. It is for this reason that primer-probe mutations are unlikely to have caused inconclusive results in our study and others (Penarrubia et al., 2020; Kim et al., 2020).

RT-PCR, serological testing including the epidemiological setting – all need to be considered in the formulation of a differential diagnosis, allowing for rapid release of accurate results and the subsequent isolation of infected patients thus limiting the spread of COVID-19 (CDC, 2020, Lou et al., 2020., Yong et al., 2020). When a negative SARS-CoV-2 RT-PCR result is obtained from symptomatic patients, a retest is advocated for and a respiratory multiplex PCR that includes viral/bacterial/fungal pathogens should be considered (Kim et al., 2020; Zhu et al., 2020).

5. Study Limitations

Our study analysed data of SARS-CoV-2 RT-PCR inconclusive results obtained only from inpatients admitted at CMJAH between June to December 2020 period as data from April to May 2020 was missing. Our study period therefore only allows for analysis of the first pandemic wave and cannot be generalizable to the period of other COVID19 pandemic waves and subsequent variants. Clinical data to confirm presence or absence of COVID- 19 symptoms and history of previous SARS-CoV-2 infection in inpatients whose samples were analysed was not available. From repeatedly inconclusive samples, retesting for SARS-CoV-2 RT-PCR on new samples, was not routinely conducted timeously.

6. Conclusion

Although SARS-CoV-2 RT- PCR inconclusive studies have been conducted in other parts of the world, our study addressed the gap of inconclusive findings in the South African public sector health context.

Despite the high sensitivity and specificity of SARS-CoV-2 RT-PCR, the occurrence of inconclusive results in the molecular laboratory is inevitable. Given the finding of 16% subsequent positivity in our study, retesting of the same sample proved to be of clinical benefit, allowing for commencement of appropriate, timeous treatment interventions resulting in improvement of patient outcomes, whilst also pivotal in the triggering of IPC measures.

Future studies on the optimisation of SARS-CoV-2 NAAT testing as well as development of algorithms are required to facilitate clinical management of SARS-CoV-2 RT-PCR inconclusive outcomes.

7. References

Ai, T., Yang, Z., Hou, H., Zhan, C., Chen, C., Lv, W., Tao, Q., Sun, Z. and Xia, L. (2020). Correlation of chest CT and RT-PCR testing for coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. *Radiology*, 296(2), pp. E32-E40.

Ardianto, C., Venna, A., Thantry, A.D.K. and Kaisar, M.M. (2022). Inconclusive results of SARS-CoV2 RT-qPCR: To retest or not? *F1000Research*, 11, p.941.

Artesi, M., Bontems, S., Göbbels, P., Franckh, M., Maes, P., Boreux, R., Meex, C., Melin, P., Hayette, M.P., Bours, V. and Durkin, K. (2020). A recurrent mutation at position 26340 of SARS-CoV-2 is associated with failure of the E gene quantitative reverse transcription-PCR utilized in a commercial dual-target diagnostic assay. *Journal of clinical microbiology*, 58(10), pp.10-1128.

Astuti, I. (2020). Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 14(4), pp.407-412.

Atkinson, B. and Petersen, E. (2020). SARS-CoV-2 shedding and infectivity. *The Lancet*, 395(10233), pp.1339-1340.

Backer, J.A., Klinkenberg, D. and Wallinga, J. (2020). Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20–28 January 2020. *Euro surveillance*, 25(5), p.2000062.

Benrahma, H., Idrissa, D., Imane, S., Jalila, R., Nida, M., Rachid, B., Khadija, A., Khadija, J., Zahra, M.F., Zahra, A. and Salma, N. (2020). Epidemiological description and analysis of RdRP, E and N genes dynamic by RT-PCR of SARS-CoV-2 in Moroccan population: Experience of the National Reference Laboratory (LNR)UM6SS. *MedRxiv*, pp.2020-06.

Bezier, C., Anthoine, G. and Charki, A. (2020). Reliability of real-time RT-PCR tests to detect SARS-Cov-2: A literature review. *International Journal of Metrology and Quality Engineering*, 11, p.13.

Bhattacharya, S., Vidyadharan, A. and Joy, V.M. (2020). Inconclusive SARS-COV-2 reverse transcription-polymerase chain reaction test reports: interpretation, clinical and infection control implications. *Journal of The Academy of Clinical Microbiologists*, 22(1), pp.59-61.

- Bhoyar, R.C., Jain, A., Sehgal, P., Divakar, M.K., Sharma, D., Imran, M., Jolly, B., Ranjan, G., Rophina, M., Sharma, S. and Siwach, S. (2021). High throughput detection and genetic epidemiology of SARS-CoV-2 using COVIDSeq next-generation sequencing. *PloS one*, 16(2), p. e0247115.
- Boeckmans, J., Cartuyvels, R., Hilkens, P., Bruckers, L., Magerman, K., Waumans, L. and Raymaekers, M. (2021). Follow-up testing of borderline SARS-CoV-2 patients by rRT-PCR allows early diagnosis of COVID-19. *Diagnostic Microbiology and Infectious Disease*, 100(2), p.115350.
- Böger, B., Ache, M.M., Vilhena, R.O., Cobre, A.F., Tonin, F.S. and Pontarolo, R. (2021). Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19. *American journal of infection control*, 49(1), pp.21-29.
- Cai, Y., Zhang, J., Xiao, T., Peng, H., Sterling, S.M., Walsh Jr, R.M., Rawson, S., RitsVolloch, S. and Chen, B. (2020). Distinct conformational states of SARS-CoV-2 spike protein. *Science*, 369(6511), pp.1586-1592.
- Carter, L.J., Garner, L.V., Smoot, J.W., Li, Y., Zhou, Q., Saveson, C.J., Sassoon, J.M., Gregg, A.C., Soares, D.J., Beskid, T.R. and Jervey, S.R. (2020). Assay techniques and test development for COVID-19 diagnosis.
- Centers for Disease Control and Prevention. (2020). Interim guidelines for collecting, handling, and testing clinical specimens for COVID-19. Accessed June 26, 2023. <https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinicalspecimens.html>
- Cento, V., Colagrossi, L., Nava, A., Lamberti, A., Senatore, S., Travi, G., Rossotti, R., Vecchi, M., Casati, O., Matarazzo, E. and Bielli, A. (2020). Persistent positivity and fluctuations of SARS-CoV-2 RNA in clinically-recovered COVID-19 patients. *Journal of Infection*, 81(3), pp. e90-e92.
- Chan, J.F.W., Yip, C.C.Y., To, K.K.W., Tang, T.H.C., Wong, S.C.Y., Leung, K.H., Fung, A.Y.F., Ng, A.C.K., Zou, Z., Tsoi, H.W. and Choi, G.K.Y. (2020). Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens. *Journal of clinical microbiology*, 58(5), pp.10-1128.
- Choudhuri, J., Carter, J., Nelson, R., Skalina, K., Osterbur-Badhey, M., Johnston, A., Goldstein, D., Paroder, M. and Szymanski, J. (2020). SARS-CoV-2 PCR cycle threshold at hospital admission associated with patient mortality. *PloS one*, 15(12), p. e0244777.
- Clerici, B., Muscatello, A., Bai, F., Pavanello, D., Orlandi, M., Marchetti, G.C., Castelli, V., Casazza, G., Costantino, G. and Podda, G.M. (2021). Sensitivity of SARS-CoV-2 detection with nasopharyngeal swabs. *Frontiers in Public Health*, 8, p.593491.
- Colton, H., Ankcorn, M., Yavuz, M., Tovey, L., Cope, A., Raza, M., Keeley, A.J., Poller, B., Parker, M., de Silva, T.I. and Evans, C. (2021). Improved sensitivity using a dual target, E and RdRP assay for the diagnosis of SARS-CoV-2 infection: Experience at a large NHS Foundation Trust in the UK. *Journal of Infection*, 82(1), pp.159-198.

Cordes, A.K. and Heim, A. (2020). Rapid random access detection of the novel SARS coronavirus-2 (SARS-CoV-2, previously 2019-nCoV) using an open access protocol for the Panther Fusion. *Journal of Clinical Virology*, 125, p.104305.

Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M.L. and Mulders, D.G. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro surveillance*, 25(3), p.2000045.

Cui, J., Li, F. and Shi, Z.L. (2019). Origin and evolution of pathogenic coronaviruses. *Nature reviews microbiology*, 17(3), pp.181-192.

Das, D. and Suresh, M.R. (2006). Copious production of SARS-CoV nucleocapsid protein employing codon optimized synthetic gene. *Journal of virological methods*, 137(2), pp.343-346.

D'Ardes, D., Boccatonda, A., Rossi, I., Pontolillo, M., Cocco, G., Schiavone, C., Santilli, F., Guagnano, M.T., Bucci, M. and Cipollone, F. (2020). Long-term positivity to SARSCoV-2: A clinical case of COVID-19 with persistent evidence of infection. *European journal of case reports in internal medicine*, 7(6).

Dhama, K., Patel, S.K., Pathak, M., Yatoo, M.I., Tiwari, R., Malik, Y.S., Singh, R., Sah, R., Rabaan, A.A., Bonilla-Aldana, D.K. and Rodriguez-Morales, A.J. (2020). An update on SARS-CoV-2/COVID-19 with particular reference to its clinical pathology, pathogenesis, immunopathology and mitigation strategies. *Travel medicine and infectious disease*, 37, p.101755.

Dolan, A.M. and FitzGerald, S. (2023). When the answer is a maybe: a retrospective review of indeterminate SARS-CoV-2 results and their associations. *Journal of Hospital Infection*.

Drew, R.J., O'Donnell, S., LeBlanc, D., McMahon, M. and Natin, D. (2020). The importance of cycle threshold values in interpreting molecular tests for SARS-CoV2. *Diagnostic microbiology and infectious disease*, 98(3), p.115130.

Dwivedy, A., Mariadasse, R., Ahmad, M., Chakraborty, S., Kar, D., Tiwari, S., Bhattacharyya, S., Sonar, S., Mani, S., Tailor, P. and Majumdar, T. (2021). Characterization of the NiRAN domain from RNA-dependent RNA polymerase provides insights into a potential therapeutic target against SARS-CoV-2. *PLoS Computational Biology*, 17(9), p. e1009384.

Engelmann, I., Alidjinou, E.K., Ogiez, J., Pagneux, Q., Miloudi, S., Benhalima, I., Ouafi, M., Sane, F., Hober, D., Roussel, A. and Cambillau, C. (2021). Preanalytical issues and cycle threshold values in SARS-CoV-2 real-time RT-PCR testing: should test results include these? *ACS omega*, 6(10), pp.6528-6536.

Falasca, F., Sciandra, I., Di Carlo, D., Gentile, M., Deales, A., Antonelli, G. and Turriziani, O. (2020). Detection of SARS-COV N2 Gene: Very low amounts of viral RNA or false positive? *Journal of Clinical Virology*, 133, p.104660.

Feinstein A.R. The inadequacy of binary models for the clinical reality of three-zone diagnostic decisions. *J. Clint. Epidemiol.* 1990; 43:109.

Fox-Lewis, A., Fox-Lewis, S., Beaumont, J., Drinković, D., Harrower, J., Howe, K., Jackson, C., Rahnama, F., Shilton, B., Qiao, H. and Smith, K.K. (2021). SARS-CoV-2 viral load dynamics and real-time RT-PCR cycle threshold interpretation in symptomatic non-hospitalised individuals in New Zealand: a multicentre cross sectional observational study. *Pathology*, 53(4), pp.530-535.

Freire-Paspuel, B., Bruno, A., Orlando, A. and Garcia-Bereguian, M.A. (2021). Analytical and clinical evaluation of two RT-qPCR SARS-CoV-2 diagnostic tests with emergency use authorization in Ecuador. *The American Journal of Tropical Medicine and Hygiene*, 104(5), p.1672.

Ganesh, B., Rajakumar, T., Maltha, M., Manikandan, N., Nagaraj, J., Santhakumar, A., Elangovan, A. and Malik, Y.S. (2021). Epidemiology and pathobiology of SARSCoV-2 (COVID-19) in comparison with SARS, MERS: An updated overview of current knowledge and future perspectives. *Clinical epidemiology and global health*, 10, p.100694.

Ghoshal, U., Vasanth, S. and Tejan, N. (2020). A guide to laboratory diagnosis of Corona Virus Disease-19 for the gastroenterologists. *Indian Journal of Gastroenterology*, 39, pp.236-242.

Gordon, D. E., Jang, G. M., Bouhaddou, M., Xu, J., Obernier, K., White, K. M., et al. (2020). A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* 583, 459–468. doi: 10.1038/s41586-020-2286-9.

Green, D.A., Zucker, J., Westblade, L.F., Whittier, S., Rennert, H., Velu, P., Craney, A., Cushing, M., Liu, D., Sobieszczyk, M.E. and Boehme, A.K. (2020). Clinical performance of SARS-CoV-2 molecular tests. *Journal of clinical microbiology*, 58(8), pp.10-1128.

Grifoni, A., Sidney, J., Zhang, Y., Scheuermann, R.H., Peters, B. and Sette, A. (2020). A sequence homology and bioinformatics approach can predict candidate targets for immune responses to SARS-CoV-2. *Cell host & microbe*, 27(4), pp.671-680.

Guan, W.J., Ni, Z.Y., Hu, Y., Liang, W.H., Ou, C.Q., He, J.X., Liu, L., Shan, H., Lei, C.L., Hui, D.S. and Du, B. (2020). Clinical characteristics of coronavirus disease 2019 in China. *New England journal of medicine*, 382(18), pp.1708-1720.

Gubbay, J.B., Rilkoff, H., Kristjanson, H.L., Forbes, J.D., Murti, M., Eshaghi, A., Broukhanski, G., Corbeil, A., Fittipaldi, N., Hopkins, J.P. and Kristjanson, E. (2022). Impact of coronavirus disease 2019 (COVID-19) pre-test probability on positive predictive value of high cycle threshold severe acute respiratory coronavirus virus 2 (SARS-CoV-2) real-time reverse transcription polymerase chain reaction (RT-PCR) test results. *Infection Control & Hospital Epidemiology*, 43(9), pp.1179-1183.

Habibzadeh, P., Mofatteh, M., Silawi, M., Ghavami, S. and Faghihi, M.A. (2021). Molecular diagnostic assays for COVID-19: an overview. *Critical reviews in clinical laboratory sciences*, 58(6), pp.385-398.

Harcourt, J., Tamin, A., Lu, X., Kamili, S., Sakthivel, S.K., Murray, J., Queen, K., Tao, Y., Paden, C.R., Zhang, J. and Li, Y. (2020). Severe acute respiratory syndrome coronavirus 2 from patient with coronavirus disease, United States. *Emerging infectious diseases*, 26(6), p.1266.

Haque, S.M., Ashwaq, O., Sarief, A. and Azad John Mohamed, A.K. (2020). A comprehensive review about SARS-CoV-2. *Future Virology*, 15(9), pp.625-648.

Hiscox, J.A., Cavanagh, D. and Britton, P. (1995). Quantification of individual subgenomic mRNA species during replication of the coronavirus transmissible gastroenteritis virus. *Virus research*, 36(2-3), pp.119-130.

Hong, K.H., Lee, S.W., Kim, T.S., Huh, H.J., Lee, J., Kim, S.Y., Park, J.S., Kim, G.J., Sung, H., Roh, K.H. and Kim, J.S. (2020). Guidelines for laboratory diagnosis of coronavirus disease 2019 (COVID-19) in Korea. *Annals of laboratory medicine*, 40(5), p.351.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X. and Cheng, Z. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet*, 395(10223), pp.497-506.

Hur, K.H., Park, K., Lim, Y., Jeong, Y.S., Sung, H. and Kim, M.N. (2020). Evaluation of four commercial kits for SARS-CoV-2 real-time reverse-transcription polymerase chain reaction approved by emergency-use-authorization in Korea. *Frontiers in Medicine*, 7, p.521.

Ishige, T., Murata, S., Taniguchi, T., Miyabe, A., Kitamura, K., Kawasaki, K., Nishimura, M., Igari, H. and Matsushita, K. (2020). Highly sensitive detection of SARS-CoV-2 RNA by multiplex rRT-PCR for molecular diagnosis of COVID-19 by clinical laboratories. *Clinica Chimica Acta*, 507, pp.139-142.

Jayakody, H., Kiddle, G., Perera, S., Tisi, L. and Leese, H.S. (2021). Molecular diagnostics in the era of COVID-19. *Analytical Methods*, 13(34), pp.3744-3763.

Katz, A.P., Civantos, F.J., Sargi, Z., Leibowitz, J.M., Nicolli, E.A., Weed, D., Moskovitz, A.E., Civantos, A.M., Andrews, D.M., Martinez, O. and Thomas, G.R. (2020). False positive reverse transcriptase polymerase chain reaction screening for SARS-CoV-2 in the setting of urgent head and neck surgery and otolaryngologic emergencies during the pandemic: Clinical implications. *Head & neck*, 42(7), pp.1621-1628.

Kim, A., Lee, H., HUR, K.W., Sung, H. and Kim, M.N. (2020). Causes and clinical relevance of inconclusive SARS-CoV-2 real-time reverse transcription PCR test results. *Annals of Clinical Microbiology*, pp.251-259.

Konrad, R., Eberle, U., Dangel, A., Treis, B., Berger, A., Bengs, K., Fingerle, V., Liebl, B., Ackermann, N. and Sing, A. (2020). Rapid establishment of laboratory diagnostics for the novel coronavirus SARS-CoV-2 in Bavaria, Germany, February 2020. *Eurosurveillance*, 25(9), p.2000173.

Lai, C.C., Wang, C.Y., Ko, W.C. and Hsueh, P.R. (2021). In vitro diagnostics of coronavirus disease 2019: Technologies and application. *Journal of Microbiology, Immunology and Infection*, 54(2), pp.164-174.

Ladhani, S.N. (2020). Prospective active national surveillance of preschools and primary schools for SARS-CoV-2 infection and transmission in England, June 2020. *SARS-CoV-2 Infection and Transmission in Primary Schools in England in June–December*.

La Jeon, Y., Lee, S.G., Lee, E.H., Song, S., Go, U.Y. and Chun, G.Y. (2023). Different interpretations of inconclusive results of SARS-CoV-2 real-time RT PCR. *Diagnostic Microbiology and Infectious Disease*, 106(2), p.115888.

Lauer, S.A., Grantz, K.H., Bi, Q., Jones, F.K., Zheng, Q., Meredith, H.R., Azman, A.S., Reich, N.G. and Lessler, J. (2020). The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Annals of internal medicine*, 172(9), pp.577-582.

Li, F. (2016). Structure, function, and evolution of coronavirus spike proteins. *Annu. Rev. Virol.* 3, 237–261.

Lim, Y.K., Kweon, O.J., Kim, H.R., Kim, T.H. and Lee, M.K. (2021). Clinical and epidemiologic characteristics of inconclusive results in SARS-CoV-2 RT-PCR assays. *BMC Infectious Diseases*, 21(1), pp.1-8.

Lin, L., Zhao, Y., Chen, B. and He, D. (2022). Multiple COVID-19 waves and vaccination effectiveness in the United States. *International journal of environmental research and public health*, 19(4), p.2282.

Lippi, G., Simundic, A.M. and Plebani, M. (2020). Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID19). *Clinical Chemistry and Laboratory Medicine (CCLM)*, 58(7), pp.1070-1076.

Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., Ren, R., Leung, K.S., Lau, E.H., Wong, J.Y. and Xing, X. (2020). Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. *New England journal of medicine*, 382(13), pp.1199-1207.

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N. and Bi, Y. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The lancet*, 395(10224), pp.565-574.

Mallett, S., Allen, A.J., Graziadio, S., Taylor, S.A., Sakai, N.S., Green, K., Suklan, J., Hyde, C., Shinkins, B., Zhelev, Z. and Peters, J. (2020). At what times during infection is SARS-CoV-2 detectable and no longer detectable using RT-PCR-based tests? A systematic review of individual participant data. *BMC medicine*, 18(1), pp.1-17.

Mehta, N., Shah, S., Paudel, K., Chamlagain, R. and Chhetri, S. (2022). Safety and efficacy of coronavirus disease_19 vaccines in chronic kidney disease patients under maintenance hemodialysis: A systematic review. *Health Science Reports*, 5(4), p.e700.

Miller, S., Lee, T., Merritt, A., Pryce, T., Levy, A. and Speers, D. (2021). Single-point mutations in the N gene of SARS-CoV-2 adversely impact detection by a commercial dual target diagnostic assay. *Microbiology spectrum*, 9(3), pp.e01494-21.

Mousavizadeh, L. and Ghasemi, S. (2021). Genotype and phenotype of COVID-19: Their roles in pathogenesis. *Journal of Microbiology, Immunology and Infection*, 54(2), pp.159-163.

Munne, K., Bhanothu, V., Bhor, V., Patel, V., Mahale, S.D. and Pande, S. (2021). Detection of SARS-CoV-2 infection by RT-PCR test: factors influencing interpretation of results. *Virus disease*, 32(2), pp.187-189.

Nörz, D., Olearo, F., Perisic, S., Bauer, M.F., Riester, E., Schneider, T., Schönfeld, K., Laengin, T. and Lütgehetmann, M. (2021). Multicenter evaluation of a fully automated high-throughput SARS-CoV-2 antigen immunoassay. *Infectious Diseases and Therapy*, 10(4), pp.2371-2379.

O'Donoghue, S.I., Schafferhans, A., Sikta, N., Stolte, C., Kaur, S., Ho, B.K., Anderson, S., Procter, J.B., Dallago, C., Bordin, N. and Adcock, M. (2021). SARS-CoV-2 structural coverage map reveals viral protein assembly, mimicry, and hijacking mechanisms. *Molecular systems biology*, 17(9), p.e10079.

Oliveira, B.A., Oliveira, L.C.D., Sabino, E.C. and Okay, T.S. (2020). SARS-CoV-2 and the COVID-19 disease: a mini review on diagnostic methods. *Revista do Instituto de Medicina Tropical de São Paulo*, 62, p.e44.

Paules, C. I., Marston, H. D., and Fauci, A. S. (2020). Coronavirus infections-more than just the common cold. *JAMA* 323, 707–708.

Peñarrubia, L., Ruiz, M., Porco, R., Rao, S.N., Juanola-Falgarona, M., Manissero, D., López-Fontanals, M. and Pareja, J. (2020). Multiple assays in a real-time RT-PCR SARS-CoV-2 panel can mitigate the risk of loss of sensitivity by new genomic variants during the COVID-19 outbreak. *International Journal of Infectious Diseases*, 97, pp.225-229.

Pfefferle, S., Reucher, S., Nörz, D. and Lütgehetmann, M. (2020). Evaluation of a quantitative RT-PCR assay for the detection of the emerging coronavirus SARS-CoV2 using a high throughput system. *Eurosurveillance*, 25(9), p.2000152.

Pujadas, E., Ibeh, N., Hernandez, M.M., Waluszko, A., Sidorenko, T., Flores, V., Shiffrin, B., Chiu, N., Young_Francois, A., Nowak, M.D. and Paniz_Mondolfi, A.E.

(2020). Comparison of SARS-CoV-2 detection from nasopharyngeal swab samples by the Roche cobas 6800 SARS-CoV-2 test and a laboratory-developed real-time RT-PCR test. *Journal of medical virology*, 92(9), pp.1695-1698.

Rastogi, Y.R., Sharma, A., Nagraik, R., Aygün, A. and Şen, F. (2020). The novel coronavirus 2019-nCoV: its evolution and transmission into humans causing global COVID-19 pandemic. *International Journal of Environmental Science and Technology*, 17, pp.4381-4388.

Rhoads, D.D. and Pinsky, B.A. (2022). The truth about SARS-CoV-2 cycle threshold values is rarely pure and never simple. *Clinical Chemistry*, 68(1), pp.16-18.

Roche Holding, A.G. (2020). cobas SARS-CoV-2 user manual. Roche Holding AG, Basel, Switzerland.

Rondaan, C., Gard, L., Niesters, H.G., van Leer-Buter, C. and Zhou, X. (2023). COVID or no COVID: interpreting inconclusive SARS-CoV-2 qPCR results in different populations and platforms. *Journal of Clinical Virology Plus*, 3(2), p.100145.

Salzberger, B., Buder, F., Lampl, B., Ehrenstein, B., Hitzenbichler, F., Holzmann, T., Schmidt, B. and Hanses, F. (2021). Epidemiology of SARS-CoV-2. *Infection*, 49, pp.233-239.

Schizas, N., Michailidis, T., Samiotis, I., Patris, V., Papakonstantinou, K., Argiriou, M. and Dedeilias, P. (2020). Delayed diagnosis and treatment of a critically ill patient with infective endocarditis due to a false-positive molecular diagnostic test for SARS-CoV2. *The American Journal of Case Reports*, 21, pp. e925931-1.

Smithgall, M.C., Scherberkova, I., Whittier, S. and Green, D.A. (2020). Comparison of Cepheid Xpert Xpress and Abbott ID Now to Roche cobas for the rapid detection of SARS-CoV-2. *Journal of Clinical Virology*, 128, p.104428.

So, M.K., Chung, H.S., Lee, D.H. and Lee, M. (2022). How Significant Are Xpert Xpress SARS-CoV-2 Test Findings When Only an N2 Gene Is Detected? *Diagnostics*, 12(9), p.2133.

Stoykova, Z., Kostadinova, T., Todorova, T., Niyazi, D., Bozhkova, M., Bizheva, S. and Stoeva, T. (2022). Dealing with inconclusive SARS-CoV-2 PCR samples—our experience. *PloS one*, 17(5), p. e0268187.

Sung, H., Roh, K.H., Hong, K.H., Seong, M.W., Ryoo, N., Kim, H.S., Lee, J., Kim, S.Y., Yoo, S., Kim, M.N. and Han, M.G. (2020). COVID-19 molecular testing in Korea: practical essentials and answers from experts based on experiences of emergency use authorization assays. *Annals of Laboratory Medicine*, 40(6), pp.439-447.

Tahamtan, A. and Ardebili, A. (2020). Real-time RT-PCR in COVID-19 detection: issues affecting the results. *Expert review of molecular diagnostics*, 20(5), pp.453-454.

Tang, X., Wu, C., Li, X., Song, Y., Yao, X., Wu, X., Duan, Y., Zhang, H., Wang, Y., Qian, Z. and Cui, J. (2020). On the origin and continuing evolution of SARS-CoV2. *National science review*, 7(6), pp.1012-1023.

Tang, Y.W., Schmitz, J.E., Persing, D.H. and Stratton, C.W. (2020). Laboratory diagnosis of COVID-19: current issues and challenges. *Journal of clinical microbiology*, 58(6), pp.10-1128.

Tombuloglu, H., Sabit, H., Al-Suhaimi, E., Al Jindan, R. and Alkharsah, K.R. (2021). Development of multiplex real-time RT-PCR assay for the detection of SARS-CoV2. *Plos one*, 16(4), p. e0250942.

Toptan, T., Hoehl, S., Westhaus, S., Bojkova, D., Berger, A., Rotter, B., Hoffmeier, K., Cinatl Jr, J., Ciesek, S. and Widera, M. (2020). Optimized qRT-PCR approach for the detection of intra-and extra-cellular SARS-CoV-2 RNAs. *International journal of molecular sciences*, 21(12), p.4396.

ul Qamar, M.T., Alqahtani, S.M., Alamri, M.A. and Chen, L.L. (2020). Structural basis of SARS-CoV-2 3CLpro and anti-COVID-19 drug discovery from medicinal plants. *Journal of pharmaceutical analysis*, 10(4), pp.313-319.

U.S. Food and Drug Administration. In Vitro Diagnostics EUAs. (2021). <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19emergency-use-authorizations-medical-devices/vitro-diagnostics-euas#individualmolecular>.

Vogels, C.B., Brito, A.F., Wyllie, A.L., Fauver, J.R., Ott, I.M., Kalinich, C.C., Petrone, M.E., Casanovas-Massana, A., Catherine Muenker, M., Moore, A.J. and Klein, J. (2020). Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT-qPCR primer-probe sets. *Nature microbiology*, 5(10), pp.1299-1305.

Yang, S., Stanzione, N., Uslan, D.Z., Garner, O.B. and de St Maurice, A. (2021). Clinical and epidemiologic evaluation of inconclusive COVID-19 PCR results using a quantitative algorithm. *American Journal of Clinical Pathology*, 155(3), pp.376-380.

Yashvardhini, N., Jha, D.K. and Bhattacharya, S. (2021). Identification and characterization of mutations in the SARS-CoV-2 RNA-dependent RNA polymerase as a promising antiviral therapeutic target. *Archives of Microbiology*, 203(9), pp.54635473.

Ye, Z.W. (2020) Zoonotic origins of human coronaviruses, *International Journal of Biological Sciences*, 16(10), p.1686.

Yong, G., Yi, Y., Tuantuan, L., Xiaowu, W., Xiuyong, L., Ang, L. and Mingfeng, H. (2020). Evaluation of the auxiliary diagnostic value of antibody assays for the detection of novel coronavirus (SARS-CoV-2). *Journal of medical virology*, 92(10), pp.1975-1979.

Coronavirus, W.H.O. (2023). WHO Coronavirus (COVID-19) Dashboard. World Health Organization. Available at <https://covid19.who.int>.

Walker, A.S., Pritchard, E., House, T., Robotham, J.V., Birrell, P.J., Bell, I., Bell, J.I., Newton, J.N., Farrar, J., Diamond, I. and Studley, R. (2021). Ct threshold values, a proxy for viral load in community SARS-CoV-2 cases, demonstrate wide variation across populations and over time. *Elife*, 10, p. e64683.

Wölfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M.A., Niemeyer, D., Jones, T.C., Vollmar, P., Rothe, C. and Hoelscher, M. (2020). Virological assessment of hospitalized patients with COVID-2019. *Nature*, 581(7809), pp.465-469.

World Health Organization. Naming the coronavirus disease (COVID-19) and the virus that causes it. [https://www.who.int/emergencies/diseases/novel-coronavirus2019/technical-guidance/naming-the-coronavirus-disease-\(covid-2019\)-and-the-virus-that-causes-it](https://www.who.int/emergencies/diseases/novel-coronavirus2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it) (Updated on February 11, 2020)

World Health Organization. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases. 2020.

World Health Organization. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases. 2020.

World Health Organization. Diagnostic testing for SARS-CoV-2. 2020.

Wu, F., Zhao, S., Yu, B., Chen, Y.M., Wang, W., Song, Z.G., Hu, Y., Tao, Z.W., Tian, J.H., Pei, Y.Y. and Yuan, M.L. (2020). A new coronavirus associated with human respiratory disease in China. *Nature*, 579(7798), pp.265-269.

Wang, Y., Kang, H., Liu, X. and Tong, Z. (2020). Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak. *Journal of medical virology*, 92(6), p.538.

Zhen, W., Smith, E., Manji, R., Schron, D. and Berry, G.J. (2020). Clinical evaluation of three sample-to-answer platforms for detection of SARS-CoV-2. *Journal of clinical microbiology*, 58(8), pp.10-1128.

Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L. and Chen, H.D. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *nature*, 579(7798), pp.270-273.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R. and Niu, P. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *New England journal of medicine*, 382(8), pp.727-733.

8. APPENDICES

Appendix 1: Data Collection Sheet

Demographic Data	Episode #			
	Specimen #			
	Date of Birth			
	Gender			
	CMJAH Ward			
Lab Information	Sample matrix	Oropharynx	☐	
		Nasopharyngeal	☐	
Date of sample receipt				
Date of sample analytical processing				
Results (Initial Inconclusive)				
TaqPath	Gene Targets & Ct values	S	ORF1a/b	N
Allplex	Gene Targets & Ct values	E	RdRP	N
Date of repeat sample processing				
Results (Repeat Inconclusive - 1)				
TaqPath	Gene Targets & Ct values	S	ORF1a/b	N
Allplex	Gene Targets & Ct values	E	RdRP	N
Date of repeat sample processing				
Results (Repeat Inconclusive - 2)				
TaqPath	Gene Targets & Ct values	S	ORF1a/b	N
Allplex	Gene Targets & Ct values	E	RdRP	N
Date of final outcome testing				
TaqPath	Gene Targets & Ct values	S	ORF1a/b	N



**NATIONAL HEALTH
LABORATORY SERVICE**

Academic Affairs and Research
Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 386 6142
Fax: +27 (0)11 386 6296
Email: babatyi.kgokong@nhls.ac.za
Web: www.nhls.ac.za

23 February 2022

Applicant: Nombuso Nxele
Institution: National Health Laboratory Service
E-mail Address: nombuso.nxele@nhls.ac.za
Tel: 011 463 9954 **Cell:** 076 948 8099

CC: Zinhle Makatini

Project Title: Repeat testing outcomes of inconclusive SARS-CoV-2 RT-PCR results in inpatients at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH)
Reference Number: PR2120527

Application Type(s):

1. Request for data

RE: APPROVAL LETTER: REQUEST TO CONDUCT RESEARCH

This letter serves to advise that an application requesting permission to conduct research has been reviewed and was **"Approved"**. Please note that approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

1. All material and data requested should be in accordance with the research protocol submitted and as approved by the relevant Health Research Ethics Committee (HREC) in South Africa.
2. Access to NHLS material and/or data will be limited to the minimum required for successful completion of the approved study and will be made available to you **without patient names**.
3. Material and/or data obtained from the NHLS shall be used anonymously and shall not for any reason be used to track or recruit patients, as no pre-approval/consent is obtained from patients.
4. Processes are discussed with the relevant NHLS departments (i.e. Corporate Data Warehouse (CDW), NHLS Laboratory Management, and Operations Office, etc.) and are agreed upon.
5. Any changes and extensions to the study requirements should be submitted for approval by an approved HREC an application submitted on the AARMS system – <https://aarms.nhls.ac.za>.
6. Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by NHLS policies.
7. The NHLS is indicted as a source of material and/or data requested in the original protocol that was approved by the (HREC) as NHLS does not have an institutional HREC.
8. A final report of the research study and any published paper resulting from this study are submitted to the NHLS AARMS or by email to academic.research@nhls.ac.za and that the NHLS has been acknowledged appropriately.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Data related queries may be directed to NHLS CDW, email: zarina.sabat@nhls.ac.za; contact number: 011 386 6074 and sample related queries (if applicable) shall be directed to the relevant business manager.

24Feb2022

Dr. Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Chairperson: Prof Eric Buch CEO: Dr Karmani Chetty
Physical Address: 1 Modderfontein Road, Sandringham, Johannesburg, South Africa Postal Address: Private Bag X8, Sandringham, 2131, South Africa
Tel: +27 (0) 11 386 6000/ 0860 00 NHLS(6457) www.nhls.ac.za
Practice number: 5200296

Enquiries: Ms N. Mzila

Email: Nolwazi.Mzila@gauteng.gov.za

Tel: 011 488 3365

Ref: 1/7/2

Date: 31 March 2023

GP_202212_038

Dr Nombuso Precious Nxele

RE: FINAL APPROVAL OF STUDY

TITLE: REPEAT TESTING OUTCOMES OF INCONCLUSIVE SARS-COV-2 RT PCR RESULTS INPATIENTS AT CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (CMJAH).

Permission is granted for you to conduct the above-mentioned study as described in your request provided:

1. Charlotte Maxeke Johannesburg Academic Hospital will not in any way incur or inherit costs as a result of the said study.
2. Your study shall not disrupt services at the study sites.
3. Strict confidentiality shall always be observed.
4. Informed consent shall be solicited from patients participating in your study.

Please liaise with the HOD and Unit Manager or Sister in charge to agree on the dates and time that would suit all parties.

Kindly forward this office with the results of your study on completion of the research.

Supported / ~~Not Supported~~

Signed by: Jayshina Punwasi
Signed at: 2023-04-03 04:44:10 +02:00
Reason: Witnessing Jayshina Punwasi



Dr J. Punwasi
Clinical Director

Approved / ~~Not Approved~~

Signed by: Gladys Magugudi Bogoshi
Signed at: 2023-04-04 11:49:53 +02:00
Reason: Witnessing Gladys Magugudi Bo



R14/49 Dr Nombuso Nxele

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M221025

NAME: Dr Nombuso Nxele
(Principal Investigator)

DEPARTMENT: Virology
Charlotte Maxeke Johannesburg Academic Hospital
National Health Laboratory Service

PROJECT TITLE: Repeat Testing Outcomes of Inconclusive SARS-CoV-2 RT
PCR results in inpatients at Charlotte Maxeke Johannesburg
Academic Hospital (CMJAH)

DATE CONSIDERED: 28/10/2022

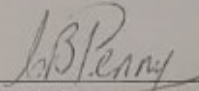
DECISION: Approved unconditionally

CONDITIONS: N/A

NOTE: N/A

SUPERVISOR: Dr Zinhle Makatini

APPROVED BY:


Dr CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL: 01/03/2023

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **October** and will therefore be due in the month of **October** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).


Principal Investigator Signature

01/03/2023
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES