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Molecular diagnostics of SARS-CoV-2: Findings of an international survey

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ABSTRACT

Background: In the current COVID-19 pandemic, early and rapid diagnosis of potentially infected and contagious individuals enables containment of the disease through quarantine and contact tracing. The rapid global expansion of these diagnostic testing services raises questions concerning the current state of the art with regard to standardization of testing and quality assessment practices. The aim of this study was to provide a global overview of the test methods, laboratory procedures and quality assessment practices used for SARS-CoV-2 diagnostics.

Methods: The Molecular Diagnostics Committee of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC C-MD) initiated a survey among international laboratories performing molecular genetic detection of SARS-CoV-2. Questions on quality assurance, variant testing, sequencing and the transmission of findings were included in the survey.

Results: A total of 273 laboratories from 49 countries participated in the survey. The majority of the participating laboratories (92.2%) use reverse transcriptase polymerase chain reaction (RT-PCR). The majority of participating laboratories do not conduct testing to identify SARS CoV-2 variants. Participation in external quality assessment programs was reported by the majority of laboratories, however, 33.2% of the laboratories reported not participating in external quality assurance programmes.

Conclusions: Based on the survey, molecular diagnostic methods for SARS-CoV-2 detection are clearly not standardized across different countries and laboratories. The survey found an array of responses in regard to sample preparation, collection, processing and reporting of results. This work suggests quality assurance is insufficiently performed by diagnostic laboratories conducting SARS-CoV-2 testing.

1. Introduction

On 11.03.2020, the WHO declared the existence of a pandemic of the Coronavirus disease 19 (COVID 19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The virus has been epidemic in over 227 countries since the outbreak in Wuhan/China and as of April 1st, 2022, almost 490 million people have been infected with SARS-CoV-2 and more than 6.1 million have died from it[2–5].

Molecular genetic detection using nucleic acid amplification tests (NAATs), such as reverse transcriptase polymerase chain reaction (RT-PCR), are the most commonly used means of detecting infection [6–13] and are also considered the standard approach for identifying an infected patient. Since the first description of the virus sequence and the publication of PCR assays for early 'in house' adoption [14], numerous

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variations of commercial and laboratory-developed-tests and -procedures (LDT/LDP) have been established [15].

PCR and other NAATs are used in the context of the ongoing pandemic for numerous reasons. They have an important role in the differential diagnostics of respiratory distress and other syndromes. Furthermore, they are used as part of the strategies to control the spread of SARS-CoV-2 and are to identify carriers of the virus. Being the most sensitive test, molecular diagnostics are performed for settings such as schools, hospitals, communal living situations (i.e., jails, prisons, nursing homes, rehabilitation facilities or community shelters), and in ambulatory care or physicians' offices.

In addition, molecular diagnostic methods are also relevant in the detection of viral mutations. According to the WHO, special attention should be paid to the Variants of Concern (VOCs) [16]. VOCs are also of interest for epidemiological reasons as they may result in changes in virulence or effectiveness of countermeasures, vaccines or therapeutics due to altered pathogen properties. As VOCs change genetically there is also a chance PCR test performance may be impacted by the emergence of a new variant and it is incumbent on diagnostic providers to be cognizant of the genetics of the variants circulating in the regions where their assays are being deployed. Currently, there are five lineages classified as VOCs with the WHO designation alpha to delta and omicron [17].

The viral variant present in a patient who tests positive is determined by molecular diagnostics either through the targeted examination of individual mutations or by determining the complete genomic sequence of the virus. The data may also be transmitted to the local health offices and authorities and is typically shared internationally via commonly used databases such as Global Initiative on Sharing Avian Influenza Data (GISAID); an initiative established for influenza that has been instrumental in supporting the sharing of SARS-CoV-2 genomics [18]).

In order to investigate the degree of standardization for SARS-CoV-2 molecular diagnostic tests at an international level and in practice, the Committee for Molecular Diagnostics of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC C-MD) conducted a voluntary web-based survey among molecular diagnostic laboratories. The data generated provide basic information on current practices in 273 molecular diagnostic laboratories from 49 countries. The results of this study intend to stimulate a broad discussion in laboratories offering molecular genetic testing for the diagnostics of SARS-CoV-2 and serve as a basis for future queries.

2. Materials and methods

The survey was designed to collect data on practices in the molecular diagnostics of SARS-CoV-2. Questions were proposed, compiled, discussed and approved by the members of the IFCC (C-MD) and were deemed important. The invitation to participate in the survey was sent out by email by the IFCC to all national societies that are members of the IFCC, starting on September 20th and ending on October 25th 2021. The survey was offered in Spanish and English. Participation in the survey was voluntary and non-traceable. The survey consisted of 29 questions, using different response formats.

The online survey addressed the following sections: 1) laboratory demographics, 2) techniques in virus detection and 3) variant testing and sequencing. The survey was designed and conducted using the software application Surveymonkey.com [3].

3. Results

3.1. Laboratory demographics

A total of 273 laboratories from 49 countries participated in the survey. The average completion time was 9 min. Participants from 197 laboratories answered the English version and 76 used the Spanish version for reporting. 105/273 (38.6%) of the laboratories were private laboratories, 18.4 % were university affiliated and 29% medical centre affiliated (79/273). Large commercial laboratories performing more than 1000 molecular genetic tests per month were 6.6% of surveyed participants.

The majority of the participating laboratories were located in Europe (n = 113) followed by Latin America (n = 68) and Asia (n = 54). Twenty-one were located in Africa and nine additional laboratories in North America. The exact origin of the participating laboratories is listed in the supplemental table 1.

The throughput of molecular diagnostic SARS-CoV-2 tests performed per week by the laboratories, showed a very wide distribution. While 17.8% of laboratories performed between 0 and 100 tests/week, 7.8% of laboratories performed more than 7000 tests/week. The majority of laboratories (31.1%) carried out between 100 and 700 tests/week, with 26.6% of laboratories realizing 1400–7000 tests/week (see Fig. 1).

3.2. Molecular diagnostic techniques used in SARS-CoV-2 detection

Regarding the molecular diagnostic methods used to detect SARS-CoV-2, 92.2% (249/270) of the laboratories reported to perform PCR. Many laboratories reported using real time RT-PCR sometimes referred to as qRT-PCR. In order to avoid implying that all laboratories were performing quantitative PCR, the term RT-PCR was used for the purpose of being more general in descriptions and discussions. Seven percent of laboratories used loop mediated Isothermal amplification (LAMP)-based techniques. Transcription mediated Amplification (TMA) was only used in 4.44%, multiplex-PCR with additional targets (i.e., Influenza A and B) in 23.3%. Direct PCR (PCR directly from samples without prior RNA purification) was reported in 10.7% while 14% of the participating laboratories run point of care test (POCT)-PCR assays (see Table 1a).

Nasopharyngeal swabs were the most frequently analyzed specimen with a frequency of 94.1% (255/271). Few laboratories (3%) reported analyzing alternate specimen types such as broncho alveolar lavages (BAL), sputum/pharyngeal washes and saliva (see Table 1b).

The majority of laboratories (81.3% =217/267) indicated that they do not heat patient samples to reduce the risk of virus exposure to laboratory personnel. Less than one-fifth of laboratories (18.7%) heat inactivate the sample before further processing and only 8.6% of the laboratories use temperatures higher than 65 °C (see Table 1c).

Various genes were targeted for the molecular detection of the virus. The nucleocapsid (N) gene of the virus was amplified in 77.3% of cases, the envelop (E) gene in 55.8%, the RNA dependent RNA polymerase (RdRp) gene in 48%, and the spike (S) gene was detected in 33.5% of cases. A small number of laboratories (7.1%), did not report or know which gene was amplified in the diagnostic procedure (see Table 1d).

The majority of the participating laboratories (i.e., 127/234 [54.3%]) reported using at least two different test procedures, while 29.1% reported using three test procedures in the SARS-CoV2 diagnostic workflow.

In regard to automation of the procedures, a majority of the laboratories (84.39%) reported automation of at least some part of the procedure. 48% of the the laboratories reported automating RNA isolation and NAAT set-up, whereas 22% of the laboratories reported using only manual procedures. (Table 1e).

In the survey, it became clear that 61.8% of the laboratories report findings electronically. Additionally, 29.0% of the participants send the results via email and 4.25% by post (see Table 1f).

In the case of a positive detection, 51.7% of the surveyed laboratories indicated that they report Cycle threshold (Ct) values and 57.7% indicated that they only report qualitative results (Positive/Detected versus Negative/Not Detected). Only 4.5% of the participants indicated reporting the number of detected copies/ml (see Table 1g). Surveyed laboratories were also asked to indicate the extent to which infectivity information is provided based on laboratory findings. Of responding laboratories, 62.1% of the laboratories do not while 37.9% do provide estimates of infectivity on their report.

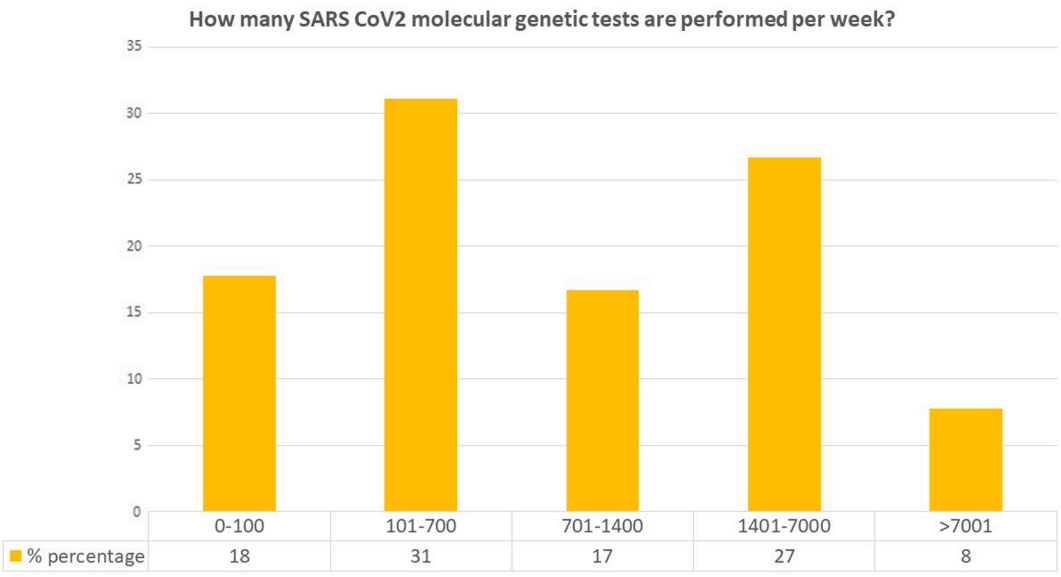

Fig. 1. Throughput of molecular genetic SARS-CoV2 tests/week at the participating laboratories. The bars show the percentage distribution.

Table 1a

What molecular genetic methods are used? (e.g. rt-PCR? and/or LAMP?)	number	%
Answer Choices		
RT-PCR	249	92.22
LAMP (Loop-mediated isothermal amplification)	19	7.04
Transcription mediated Amplification (TMA)	12	4.44
Multiplex PCR with other targets (Influ A, Influ B, etc)	63	23.33
Direct PCR (without nucleic acid isolation)	29	10.74
POCT-PCR	38	14.07
Answered	270	
Skipped	3	

Table 1b

Which specimens are mainly examined?	number	%
Nasopharyngeal swabs	255	94.0.10
Bronchoalveolar lavage (BAL)	1	0.0.37
Sputum/pharyngeal wash	2	0.74
Saliva	7	2.58
Other	6	2.21
Answered	271	
Skipped	2	

Table1c

Are samples heated prior to processing to decrease exposure to personnel?	number	%
No.	217	81.27
Yes. We use a temperature less than 55 °C.	9	3.37
Yes. We use a temperature between 55 and 60 °C.	8	3.00
Yes. We use a temperature between 60 and 65 °C.	10	3.75
Yes. We use a temperature greater than 65 °C.	23	8.61
Answered	267	
Skipped	6	

Table 1d

Which SARS-CoV2 specific gene is targeted in the detection procedure?	number	%
N-gene	208	77.3
RdRP	129	48.0
E-gene	150	55.8
S-gene	90	33.5
I doṅt know	19	7.1
Other	61	22.7
Answered	269	
Skipped	4	

Table 1e

How are the SARS-CoV2 detections performed?	number	%
RNA isolation and PCR setup manually	60	22.30
RNA isolation automated / PCR setup manually	98	36.43
RNA isolation and PCR setup automated	129	47.96
No RNA isolation + direct PCR	41	15.24
Other	11	4.09
Answered	269	
Skipped	4	

Table 1f

How are the findings predominantly transmitted?	number	%
Fax	7	2.70
Email	75	28.96
Via direct connection/Electronic transmission of laboratory results	160	61.78
By mail/letter	11	4.25
Other	6	2.32
Answered	259	
Skipped	14	

3.3. External quality assessment (EQA)

External quality assessment (EQA) is an interlaboratory comparison that may extend throughout all phases of a testing cycle including interpretation of results. It is understandable, that characterised reference materials or external quality controls were lacking, especially at the beginning of the pandemic. In our survey, more than 18 months after the start of the pandemic, the survey asked the laboratories whether they participate in external quality controls and if so, which ones.

Many laboratories (n = 232) answered the question and 77 (32.2%) do not participate in external quality controls. Of the EQA providers mentioned, CAP [19]) and Instand e.V [20]. were the most frequently mentioned (n = 19). Eleven laboratories obtained characterised material from WHO. Eight laboratories investigate external quality control specimens from QCMD [21] (n = 8), ICMR (Indian Council of Medical

Table 1g

Which result is transmitted in a positive case?	number	%
Ct value	138	51,69
Number of detected copies/ml	12	4,49
TC50	0	0,00
Only qualitative result (pos/neg)	154	57,68
Other (please specify)	13	4,87
Answered	267	
Skipped	6	

Research) [22] or RfB (each n = 6) [23] (data not shown). In response to the question "Are reference materials of defined concentration used for test validation?", 128 (48.1%) of laboratories agreed, while 136 answered in the negative. The frequency of the use of the reference material varied greatly. While some laboratories processed control material for every run, others only used it for the quality control of a new batch of reagents. Other labs reported using it only once/year (see Table 2a). One third of laboratories (29.5%) use calibrators to define the limit of quantification (LoQ) in their SARS-CoV-2 test approach. Most laboratories (184/261, 70.5 %) do not establish the LoQ for their assays (see Table 2b). Where quantitative reporting of results used by the laboratory (n = 62) is undertaken, 16 laboratories report using one calibrator, while 23 work with two calibrators and 23 participants reported using 3 or more calibrators (Table 2c).

The majority of laboratories reported that the tests performed are validated by the laboratory doctor (167/269 = 62.1%). Technical assistants validate the generated results in 23.8% (64/269) and the head of department in 29.3% (79/269) of the cases (data not shown).

3.4. Detection and characterisation of VOCs (Variants of Concern)

An important tool in understanding the evolving nature of the COVID-19 is the characterisation of positive virus findings and for the rapid detection of so-called VOCs. Efforts for such characterization has been performed since the first description of the alpha variant genotype detection for the surveillance of mutant virus strains.

There are typical mutations described for each VOC. In genotyping, these sequence variations are used to identify the lineage of the virus detected in a specimen.

Of the surveyed laboratories 25% stated, that they genotype positive cases in their own laboratories. Most participating laboratories (201/268) do not carry out such tests. The sequence variations N501Y, E484K and L452R were most frequently mentioned by laboratories as parameters to be tested. If a VOC is detected, this information is reported to the healthcare provider in 67.8% (154/277) of the participating laboratories; in 79.65% the information is reported to a public health or governmental organization.

The current development of the SARS-CoV-2 pandemic shows a continuous emergence and spread of genetic variants of the virus with partly increased infection potential and variable pathogenicity. It is therefore necessary to monitor and research these dynamic processes of variant emergence and spread. Sequencing and bioinformatic analysis of complete viral genomes is the tool of choice for this. According to our survey, 36 (13.6%) of the 262 laboratories, that provided an answer to this question, carried out sequencing, whereby the throughput here also varied considerably between the laboratories. Between one and 100 virus sequencings per week were carried out. However, significantly more laboratories, namely 124/264 (48.1%), forward aliquots of some of their positively tested samples to external laboratories for sequencing and subsequent characterisation of the virus lineage. Here, the data ranges from one forwarded sample per week to 200 samples per week.

Most laboratories (51.9 %) do not have their samples sequenced at all.

Table 2a

Are reference materials of defined concentration used for test validation?	number	%
Yes	128	48.1
No	136	51.1
if so which and how often?	34	12.8
Answered	266	
Skipped	7	

Table 2b

Are calibrators used to determine the lower limit of quantification (LoQ)?	number	%
Yes	77	29.5
No	184	70.5
Answered	261	
Skipped	12	

Table 2c

How many calibrators are used for quantitative reporting of results?	number	%
1	16	6.18
2	23	8.88
3	14	5.41
>3	9	3.47
We do not provide quantitative reporting of results.	197	76.06
Answered	259	
Skipped		

4. Discussion

The molecular genetic diagnosis of COVID-19 is primarily based on the detection of the RNA of SARS-CoV-2. NAATs, like RT-PCR, allow the detection of specific sequences with viral genes (i.e., N-, S-, E-Gene, etc.). At the present time, molecular detection of SARS-CoV-2 using NAATs can be expected to be the most widely performed analysis in laboratory medicine although the increase in the use of antigen based methods raises an interesting question about comparability of the formats and what their analytical differences mean with regards to clinical performance.

In order to get a better understanding of the methods currently used for this purpose and to identify if differences and problems exist, the C-MD of the IFCC initiated a survey highlighting important points in the diagnostic process of SARS-CoV2 infection. 273 laboratories participated between September 20th and October 25th, thus providing the most comprehensive data on molecular genetic SARS-CoV-2 diagnostics worldwide.

The testing throughput of laboratories responding to this survey covers a wide range. Most laboratories (57.7%) perform more than 100 tests per week and every fourth laboratory (26.6%) analyze more than 200 samples per day. An important finding in the interpretation of the data is that despite a massive throughput of molecular genetic testing worldwide, extensive standardization is lacking. This concerns the pre-analytical/pre examination factors, an almost non-existent comparability of the results as well as the lack of participation in external quality controls. With such high testing volumes and the stated diversity in testing methods, it is concerning that many laboratories do not participate in EQA programs to confirm testing quality. Specifically, 77/232 laboratories stated that they did not participate in EQA testing. Fifty laboratories skipped answering this question in the survey. This may be due to a limited availability of characterized EQA material on the one hand or to the potential regional cost intensity of EQA samples on the other. Another aspect of quality assurance is also answered very differently by the participating laboratories. Fewer than half of the laboratories stated that they use samples of a defined concentration for the internal validation of molecular genetic detection.

Detection of SARS-CoV-2 RNA in nasopharyngeal swabs was the

most widely used specimen for identifying infected individuals by the surveyed laboratories, and a small minority of laboratories (4.8%) indicated using saliva or other samples as the primary specimen examined. Detection of the virus has been described on a variety of clinical specimens such as saliva and sputum and they could have benefits over nasopharyngeal swabs [1,2,3]. However, it is critical to standardize the protocols for collection of these alternative specimens and validate their use to identify infected individuals before they are widely used. It is also important to understand that the ideal specimen for viral detection will have to be continuously evaluated. This is because the viral tropism for different cell types could change as the virus keeps evolving.

Assays that detect VOCs provide an additional diagnostic output. Knowing which variant is prevalent may be relevant to disease management. However, it should be emphasized in the global survey that only about 25% of laboratories offer genotyping for variant identification at all (67/268). The clear majority of laboratories do not monitor VOCs. This may be due to limited resources (ie reagents, equipment and personnel) being directed toward diagnostic detection of SARS-CoV-2 rather than genetic characterization of the virus. Another explanation may be, that further molecular characterization by variant genotyping is potentially not additionally remunerated and therefore appears unattractive for laboratories. Comparably results are also shown for molecular monitoring by next-generation sequencing. Although numerous methodological simplifications have been made in this area, whole genome sequencing of SARS-CoV-2 for surveillance is only carried out by a fraction of the laboratories. Only 13.6% of all laboratories are able to carry out sequencing and thus provide the actual basis for molecular genetic monitoring. In context of the pandemic situation and the ongoing possibility of emerging new viral mutations, there is certainly a need to discuss improving sequencing efforts globally.

Diagnostic providers must be cognizant of the potential for genetic changes to impact their assays, monitor the potential impact and inform those using the tests of any possible impact; which at its worse can lead to a false negative of a newly spreading variant. This must be done by examining the genetics of the variants present in the region(s) where the assay is being deployed.

The rapid detection of the Omicron variant is certainly a successful example of very efficient detection of a VOC. Further studies that address the sequencing capacities and performance of the laboratories are certainly necessary.

A limitation of the present study is that participation was possible only for laboratories whose national professional societies are members of the IFCC. In addition, it should be mentioned that genotyping for variants is potentially region-specific. For example, if the Delta variant is not analyzed in a country at the time of the study, it is understandable that the variants that may be indicative of the Delta variant is correspondingly underreported by the laboratories in our survey.

Another limitation is that some countries were not well represented in the study. Only a few contacted laboratories from Central or South Africa, from China or from Oceania participated in the survey.

At the present time, molecular genetic detection of SARS-CoV-2 using NAATs is the most widely performed diagnostic format in laboratory medicine. However, the increase in the use of antigen-based methods raises an interesting question about comparability of the formats and what their analytical differences mean with regards to clinical performance. How these respective formats are supported in terms of quality assurance remains an important question as our efforts to identify SARS-CoV-2 become more advanced and we begin to better understand and live with COVID-19 globally.

CRediT authorship contribution statement

Aldo Vacaflores Salinas: Conceptualization, Writing – original draft, Writing – review & editing. **Tester Ashavaid:** Conceptualization, Writing – original draft, Writing – review & editing. **Deborah A. Payne:** Conceptualization, Writing – original draft, Writing – review & editing. **Mark W. Linder:** Conceptualization, Writing – original draft, Writing – review & editing. **Katarina Baluchova:** Writing – review & editing. **Shiyang Pan:** Writing – original draft, Writing – review & editing. **Jim Huggett:** Conceptualization, Writing – original draft, Writing – review & editing. **Parviz Ahmad-Nejad:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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Competing interests

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Appendix A. Supplementary material

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