LABORATORY METHODS AND PREVALENCE OF SARS-COV-2 INFECTIONS IN THE 2ND SEMESTER OF 2021 IN THE EMERGENCY CLINICAL COUNTY HOSPITAL OF CONSTANTA

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ABSTRACT

Diagnosing infections with SARS-CoV-2 is still of great interest due to the health and economic impact of COVID pandemic. The 4th wave of the COVID-19 pandemic is expected and is considered to be stronger and faster due to the dominance of Delta variant which is highly contagious [1].

SARS-CoV-2 also known as 2019-nCoV is one of the three coronaviruses (together with SARS-CoV or SARS-CoV1/Severe acute respiratory syndrome coronavirus), MERS-CoV /Middle East Respiratory Syndrome coronavirus) which can cause severe respiratory tract infections in humans [2].

Early diagnosis in COVID 19 infection is the key for preventing infection transmission in collectivity and proper medical care for the ill patients.

Gold standard for diagnosing SARS-Co-V-2 infection according to WHO recommendation is using nucleic acid amplification tests (NAAT)/ reverse transcription polymerase chain reaction (RT-PCR).

The search is on to develop reliable but less expensive and faster diagnostic tests that detect antigens specific for SARS-CoV-2 infection. Antigen-detection diagnostic tests are designed to directly detect SARSCoV-2 proteins produced by replicating virus in respiratory secretions so-called rapid diagnostic tests, or RDTs.

The diagnostic development landscape is dynamic, with nearly a hundred companies developing or manufacturing rapid tests for SARS-CoV-2 antigen detection [3].

In the last 3 months our hospital introduced the antigen test or Rapid diagnostic tests (RDT) which detects the presence of viral proteins (antigens) expressed by the COVID-19 virus in a sample from the respiratory tract of a person. All RDT were confirmed next day with a RT-PCR.

The number of positive cases detected during 3 months in our laboratory was 425. There were 326 positive tests in April, 106 positive tests in May and 7 positive tests in June. Compared with the number of positive tests in the 1st semester of 2021, the positive tests have significantly declined.

Keywords: *SARS-CoV-2, real time-RT PCR, Antigen, Rapid diagnostic tests*

INTRODUCTION

Diagnostic tests have been considered of paramount importance for the control of coronavirus disease (COVID-19). Diagnostic is even more important as the 4th wave of COVID 19 pandemic is expected.

Coronaviruses are enveloped, non-segmented, positive-sense, single-stranded RNA viruses.

SARS-CoV-2 possess 16 non-structural proteins (such as RNA dependent RNA polimerase RdRp) encoded by ORFs: ORF1a, ORF 1b and structural proteins (encoded within the 3′ end of the viral genome) including membrane (M), envelope (E), spike (S), and nucleocapsid (N) proteins [4].

The S glycoprotein is a class I fusion protein and directs attachment to the host receptor. It is formed by functional subunits, S1 and S2. Subunit S1 is formed by N terminal domain (NTD) and Receptor Binding Domain (RBD).

Subunit S2 contains fusion peptide (FP), heptad repeat 1 (HR1), central helix (CH), connector domain (CD), heptad repeat 2 (HR2), transmembrane domain (TM), cytoplasmic tail (CT). The SARS-CoV Spike fusion protein subunit S2 plays an important role in viral entry by initiating fusion of the viral and cellular membranes [5]

MATERIALS AND METHODS

Diagnostic tests for COVID-19 can be organized in virological diagnosis, recommended for virus detection, and serological tests, recommended for assessing the disease progression/development of immune response.

Virological diagnoses used in our laboratory were RNA amplification-based detection methods/ real-time RT-PCR, and viral proteins detection assays/ Rapid Diagnostic tests (RDT).

RT- PCR is an in vitro diagnostic real-time reverse transcription-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal/oropharyngeal swabs, anterior/mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. [6]

The assay detects three SARS-CoV-2 RNA targets: the envelope (E) gene, the nucleocapsid (N) gene and a region of the open reading frame (ORF1) of the RNA dependent RNA polymerase (RdRp) gene from SARS-CoV-2 virus isolate Wuhan-Hu-1.

First step is represented by RNA extraction from samples. Once RNA is extracted the eluates are amplified. The target RNA is converted to cDNA by the reverse transcriptase enzyme. The obtained cDNA is amplified by PCR. Amplification takes place in a thermal cycler. Each cycle of PCR includes steps for template denaturation (94°C), primer annealing (40–60°C) and primer extension (70–74°C). [7]

WHO recommends using nucleic acid amplification tests (NAAT)/ reverse transcription polymerase chain reaction (RT-PCR) for diagnosing SARS-Co-V-2 infection as a gold standard.

RT-PCR testing is limited to certified Clinical Laboratory/Molecular Biology laboratories which require trained personnel, specific chemical supplies and expensive instruments. This can limit the number of tests that can be done.

Therefore alternatives were used for a rapid result mainly in symptomatic patients which were admitted in different hospital wards.

Antigen rapid Diagnostic Tests (Ag-RDTs) detect antigens from clinical specimens using a simple-to-use immunochromatographic (lateral flow) test format.

RDTs are typically a nitrocellulose strip enclosed in a plastic cassette with a sample well. When the infected patient's sample is combined with the test buffer and added to the sample well of the test strip, target antigens in the mixture bind to labelled antibodies and migrate together; they are subsequently captured by an antibody bound to the test line, triggering a detectable colour change. Depending on the test (and the antibody labels used), the colour change can be read by the operator with or without the aid of a reader instrument [8]. RDTs for COVID-19 can produce results in around 10–30 minutes versus the many hours required for most NAATs [8].

SARS-CoV-2 Ag-RDTs are authorized for use under emergency conditions and have not undergone comprehensive and stringent regulatory review.

The RDTs used in the Emergency County Hospital of Constanta were Rapid SARS-CoV-2 Antigen test card from Xiamen Boson and SD Biosensor COVID-19 Ag Test.

WHO recommends that a small set of samples from SARS-CoV-2 NAAT-confirmed positive and negative samples may be tested in parallel to assess whether the new kit meets performance requirements. In our lab we re-tested all Ag-RTD with RT-PCR with a very good correlation.

RESULTS AND DISCUSSIONS

Our study was made in the 2nd trimester of year 2021 using the real time RT PCR and Ag- RTD. The laboratory is testing the inpatients from the hospital wards and the hospital employees.

During first 3 months of 2021 (January, February, March) were tested with RT-PCR and confirmed positive a number of 993 patients and 83 health care personnel and other professional workers in hospital.

In the 2nd semester of 2021 (April, May, June), the total number of positive tests has significantly decreased. There were 425 positive tests for patients and 14 positive tests for hospital staff. Tests were made using RT-PCR and Ag-RDTs confirmed with RT-PCR.

The distribution of positive cases in the 2nd trimester of 2021 with relation to the month and the hospital wards (for inpatients, outpatients) and hospital staff workplace is presented in the next tabel.

Month	**April**	**April**	**May**	**May**	**June**	**June**
Ward	Health care personnel	Patients	Health care personnel	Patients	Health care personnel	Patients
Emergency ward	0	249	0	59	0	3
Hemodialysis	0	3	0	0	0	0
Oncology	0	8	0	2	0	0
Internal medicine 1	0	6	0	5	0	1
Internal medicine 2	0	3	0	1	0	1
Nephrology	0	0	0	1	0	0
Neurology	1	4	1	6	0	0
Gastroenterology	0	1	0	1	0	0
ICU	0	3	0	2	0	0
Cardiology	2	16	1	11	0	2
Orthopedics	1	1	0	0	0	0
Op theater orthopedics	0	0	0	0	0	0
Cardiovascular surgery	0	1	0	1	0	0
Surgery ward 2	0	1	0	0	0	0
Surgery ward 1	0	1	0	1	0	0
Urology	0	2	0	0	0	0
Ob/ gynecology ward 2	3	0	0	0	0	0
Ob/gynecology ward 1	0	0	0	0	0	0
Neurosurgery ward	0	0	0	8	0	0
Otorhinolaryngology	0	1	0	0	0	0
Pediatrics	0	11	0	4	0	0
Pediatric surgery	0	0	0	0	0	0

Psychiatry	0	2	0	0	0	0
Osteo-articular tuberculosis	0	0	0	0	0	0
Palliative care	0	0	0	0	0	0
Physical Rehabilitation	0	2	0	0	0	0
Dermatology	0	0	0	0	0	0
Pneumo-phthisiology	0	0	0	0	0	0
Radiotherapy	0	0	0	1	0	0
Forensic medicine	0	0	1	0	0	0
Hospital Technical staff	0		0		0	
Hospital administration	0		0		0	
Medical front desk clerk	0		0		0	
Hospital housekeeper staff	4		0		0	
TOTAL	**11**	**315**	**3**	**103**	**0**	**7**

CONCLUSIONS

The Cellular Biology laboratory of the University Emergency Clinical Hospital is testing for SARS-CoV-2 infections using RT-PCR technique. Due to emergency needs during night shifts, patients are tested in the emergency ward using Antigen RDTs. All tested patients using rapid antigen tests are retested the next day with RT-PCR.

During 2nd trimester of 2021 were performed 2998 RT-PCR tests (1375 in April, 988 in May, 635 in June) were with 425 positive results (326 positive tests in April, 106 positive tests in May and 7 positive tests in June). The positivity rate was 23.70% in April, 10.72% in May, 1.10% in June.

There was a strong decline in the prevalence of SARS-CoV-2 infection.

The highest positivity rate was for the patients from the Emergency ward.

There were few cases among health care personnel due to prophylactic measures such as equipment and vaccination and also by natural immunization after infection. There is no routine screening for antibody seroprevalence among healthcare workers.

RT-PCR detects active SARS-CoV-2 infection and has high sensitivity and specificity but are expensive, requires laboratory infrastructure and skilled personnel, with a turnaround time of hours or days.

Ag RDT detects active SARS-CoV-2 infection, easy to perform, quick results enabling rapid implementation of infection control measures, less expensive than RT-PCR, but with variable sensitivity and specificity. Lower sensitivity means the

negative predictive value is lower than for RT-PCR. Confirmatory RT-PCR of Ag-RDT positive is required in low prevalence settings and for Ag-RDT negative in high prevalence settings. Negative Ag-RDT cannot be used to remove a contact from quarantine [9].

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