# STUDY ON THE INCIDENCE OF ANTI-SARS COV2 ANTIBODIES TO HEALTHCARE STAFF

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## INTRODUCTION

The Coronavirus 2019 disease (Covid-19) is characterized by severe acute respiratory coronavirus syndrome: SARS CoV2, which was first identified in Wuhan city and Hubei in China.

In March 2020, the WHO admitted: COVID-19 can be characterized as a pandemic (it is an international public health emergency, there is a high risk of the disease spreading in many countries around the world). On July 7, 2020, the authorities in our country announced that since the beginning of the pandemic, a number of 3335 cases of infection have been registered among the medical staff, which means 11.4%.

The exact interval in which a person with SARS-CoV-2 infection can transmit the infection to others is uncertain, the potential for transmission begins before symptoms occur when the risk of transmission is very high, and after 7 to 10 days of illness is unlikely, especially for otherwise immunocompetent patients with non-severe infection. Infected people are more likely to be contagious in the earlier stages of the disease, when the level of viral RNA (in the upper respiratory segments) reaches a maximum.

The new Coronavirus (Covid-19) consists of a single RNA strand. Comparison of the genetic sequence of this virus showed the similarity with SARS-CoV, but also coronaviruses from bats.

Coronaviruses are composed of several proteins that include spike protein (S), coating protein (E), membrane protein (M) and nucleocapsid protein (N). The results suggest that this spike protein has sufficient affinity for angiotensin, which causes the enzyme receptor (ACE2) to be used as a mechanism of entry into the cell.

A modeling study suggested that the infectivity started 2-3 days before the onset of symptoms, peaked at 0.7 days before the onset of symptoms, and decreased within seven days. Prolonged detection of viral RNA does not indicate prolonged infectivity. The duration of viral RNA clearance is variable and may increase with disease severity. In some people, viral RNA can be detected in the respiratory tract a few months after the initial infection. However, detectable viral RNA does not always indicate the presence of the infectious virus and there appears to be a threshold below which the infectivity is unlikely. Furthermore, the infectious virus was not detected in respiratory samples when the viral RNA level was below 106 copies/ml.

The most common symptoms are fever, dry cough, fatigue, sputum production, dyspnea or difficulty breathing, sore throat, headache. Some patients may complain of muscle aches, nausea, nasal congestion, diarrhea. Some patients may be asymptomatic.

Approximately 80% of infected patients recover from the disease without specific treatment. The elderly and those with associated diseases (hypertension, diabetes, heart problems) are prone to develop a severe form of viral pneumonia with respiratory failure that can progress to severe acute respiratory syndrome, septic shock, multiple organ failure, metabolic imbalance severe acid-base, which can be life-threatening. To date, about 4% of confirmed cases have died.

COVID-19 is transmitted mainly by direct contact with secretions or drops released by people infected by coughing, sneezing, speech, which may be inhaled or ingested directly by other people or which may contaminate surfaces. The disease can also be transmitted by fecal-oral route. The incubation period of the virus varies between 1-14 days. Measures to prevent and limit the transmission of the disease are essential in both healthcare and the community. These measures have been and are long publicized. Medical personnel must apply additional precautions to protect themselves and prevent transmission during medical care.

## MATERIAL AND METHOD

a) Preparation of the biological product to be analyzed

Serum samples are made from primary tubes that have have barcodes corresponding to the patient ID. Serum samples that cannot be processed on the same day will be transferred to plastic tubes, the minimum amount is 500 μl and stored in the freezer -20 C for 2 months. After thawing, the samples will be

centrifuged and transferred to 2 ml ependorf tubes for work on the analyzer. Defrosted samples are worked only once. Do not work samples that have been frozen/thawed repeatedly.

A number of 224 was tested from health personnel from the departments of ATI, gastroenterology, adult infectious diseases, obstetrics-gynecology, nephrology, medical analysis laboratory.

b) Method

The DYNEX automated analyser, produced by Dynex Technologies Inc, is an open automated system that can process ELISA analyzes on microplates. All operations are automatic and programmable. Reagent kits used for antibody testing are provided by Diamedix. These kits can be used to monitor the antibody response after SARS CoV2 infection or to study the condition of the antibodies as a screening test.

**The principle of the Covid IgM and IgG test**:

Covid IgM and IgG are solid enzyme-linked immunosorbent assays (ELISA) for the determination of the IgM and IgG antibodies anti-CoV-19 in serum and plasma. The diluted samples will be pipetted into antigen-coated wells specific to the spike protein and specific to the virus nucleocapsid. Neutralizing reagent is added to block interference that may occur due to rheumatoid factor. IgM, IgG antibodies if present are captured by antigens in the wells. After washing, the conjugate is added (polyclonal antibodies obtained from goat's blood marked with horseradish peroxidase), the antigen-antibody complex is formed. After incubation, wash and add the substrate (TMB). At the end of the incubation, the reaction is stopped and the OD (optical density) is read.

## RESULTS AND DISCUSSIONS

A number of 224 was tested, of which 45 came from males and 179 from females, the sex ratio being: F/M = 3.98 (Fig. no. 1). The age of the patients ranged from 23 to 62 years.

The analysis of Fig. No. 1 shows a higher number of females investigated compared males, which is also explained by the fact that the share of female health personnel in hospitals is much higher than that of male staff.

Following the serological determinations on the Dynex analyzer, 80 reactive samples were obtained, of which 67 came from females (83.75%) and 13 from males (16.25%). Of the 80 people detected reactive, 60 of the people went through the disease a few months ago, the remaining 20 people did not show symptoms specific to Covid 19 infection, these sera were repeated on DIAPRO kits, the values obtained being similar, it is necessary performing alternative methods for antibody detection.

The distribution by age groups and by sex of reactive detected personnel is show in next figure. From the analysis of Fig. 2, it is found that the highest number of reactive cases were registred in the age group 41-50 years for female staff, and for male in the group 21-30 years.

From the analysis of Fig. 3, the highest values were obtained in the age group over 60 years and the lowest values in the age group 21-30 years.

Analyzing Fig. 4, it is found that for tis category of personnel the highest values of IgG antibodies were registered in the age group 41-50 years and the age over 60 years. For IgM antibodies highest values are in group 51-60 years, respectively the group over 60 years.

According to the data in the insert of the Covid IgM DiaPRO kit, the presence or absence of anti-SAR IgM antibodies. COV-2 is determined by comparing the absorbance (OD) of each sample with the calculated value of the cut-off. The value of the cut-off (Co) is obtained from the arithmetic mean of the negative controls (NCx) + 0.25.
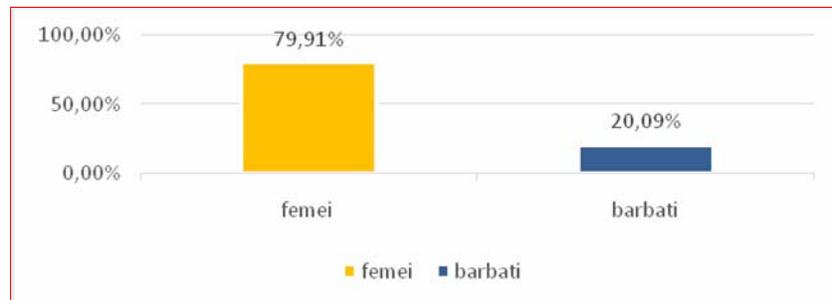


Fig. 1. Percentage distribution of the investigated personnel
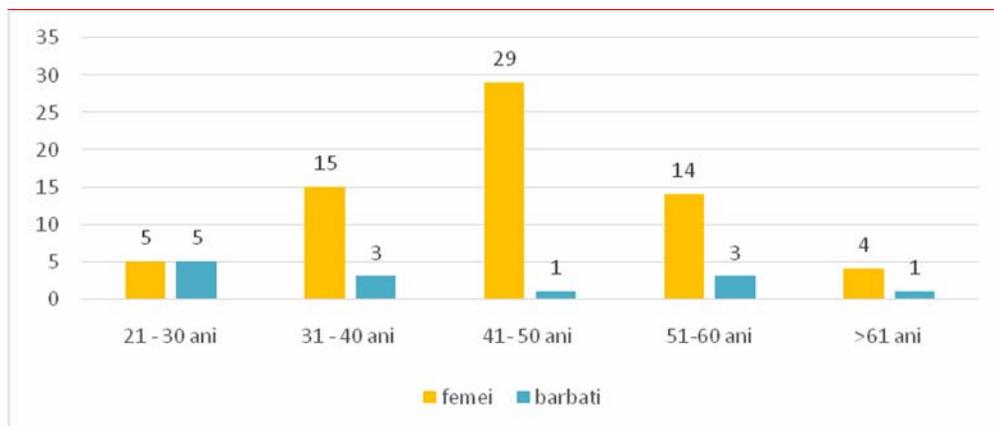
Fig. 2. Distribution by age groups and by sex of the reactive detected personnel
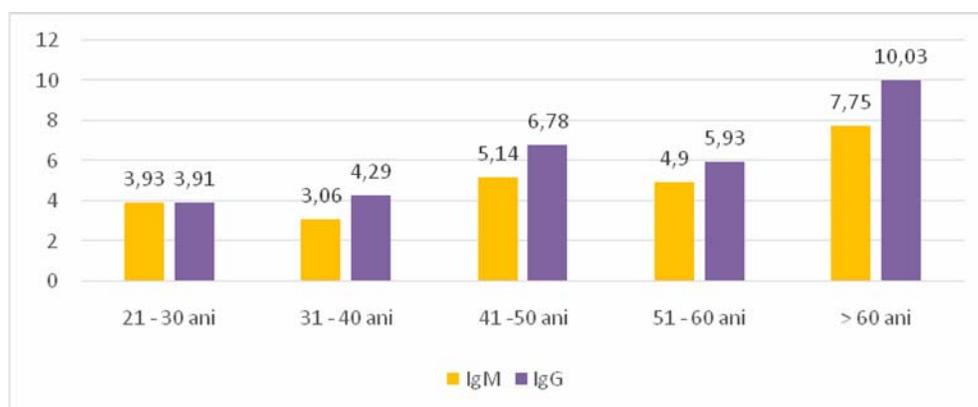


Fig. 3. Mean values of anti-SARS CoV2 IgM si anti-SARS CoV2 IgG antibodies
obtained in females
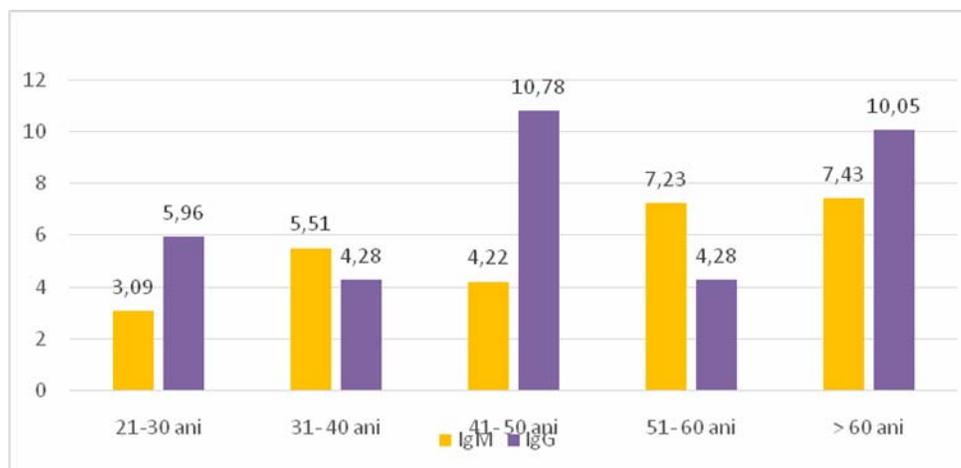


Fig. 4. Mean values of anti-SARS VOC 2 IgM antibodies and anti-SARS VOC 2 IgG antibodies obtained in
males

*Validation criteria*
For: negative control: each measured value of the absorbance of a sample must be ≤ 0.150 units of optical density; positive control: the absorbance value must be greater than or equal to 1.1; blank: the absorbance value must be < 0.100; calibrator: the absorbance value of the calibrator/Co must be less than 1.1.

Interpretation of results:
All results are interpreted according to the DO (sample)/DO(cutt-off) ratio as follows:
-Non-reactive: < 0.9;
-Uncertain: 0.9 – 1.1; Uncertain samples will be retested after two weeks requesting a new harvest.
-Weakly reactive: 1.1 – 2 for IgM and 1.1 – 3 for IgG;
-Reactive: > 2 for IgM and > 3 for IgG.
From the recorded data, IgM reactivity was as follows:
From the analysis of Fig. No. 5 it is found in femals, the same percentage of 13.43% for non-reactive

cases and weakly reactive ones regarding the presence of IgM antibodies against SARS CoV2 and a percentage of 73.14 % for reactive cases.

For male staff, the same situation is similar for female staff, an equal percentage (15.38%) of non-reactive and weakly reactive cases, but we have the situation of a 36-year-old man who showed an uncertain result for IgM antibodies. anti SARS COV 2 (7.7%) and a reactive result for anti SARS COV2 IgG antibodies. For reactive values of anti-SARS COV2 IgM antibodies in male staff, a percentage of 69.23 % was recorded.

Following the determinations of IgG antibodies in females, the percentages were obtained: 4.48 % non-reactive cases, 19.40 % weakly reactive and 76.12 % reactive.
For IgG antibodies in males only weakly reactive values (30.77 %) and reactive values (69.23 %) were obtained.
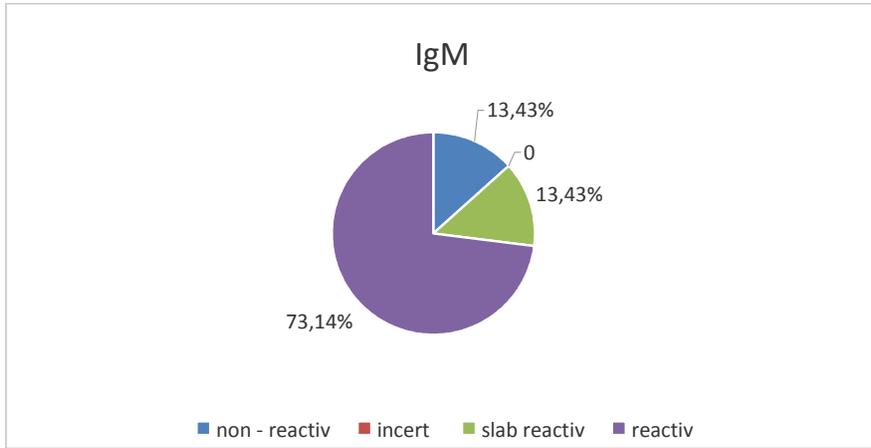


Fig. 5. Distribution of results for anti-SARS CoV2 IgM antibodies in females
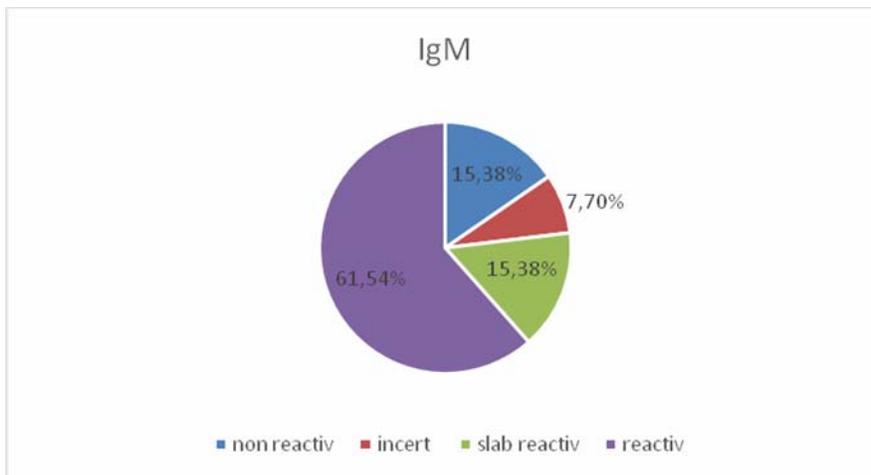


Fig. 6. Distribution of results for anti-SARS CoV2 IgM antibodies in males

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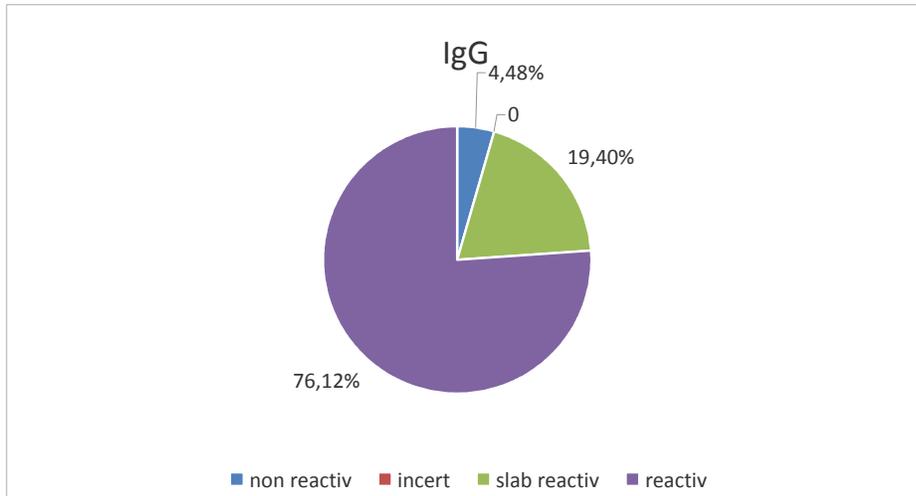
Fig. 7. Distribution of results for anti-SARS CoV2 IgG antibodies females
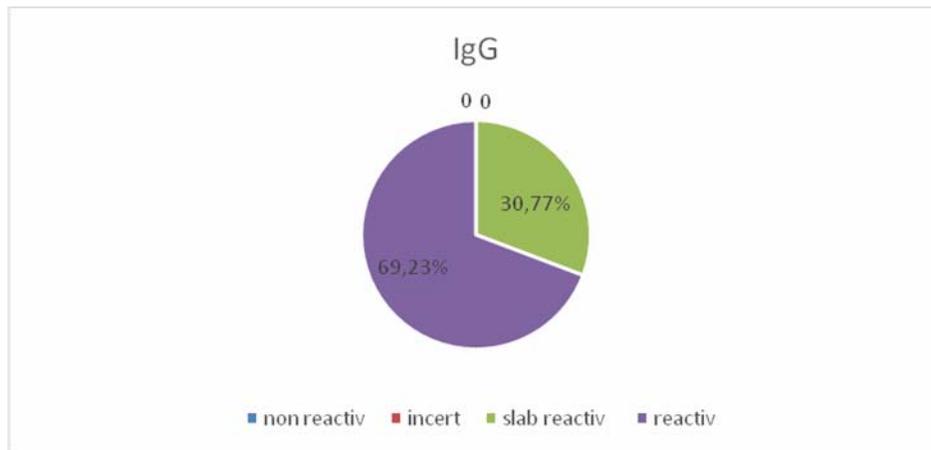


Fig. 8. Distribution of results for anti-SARS CoV2 IgG antibodies in males
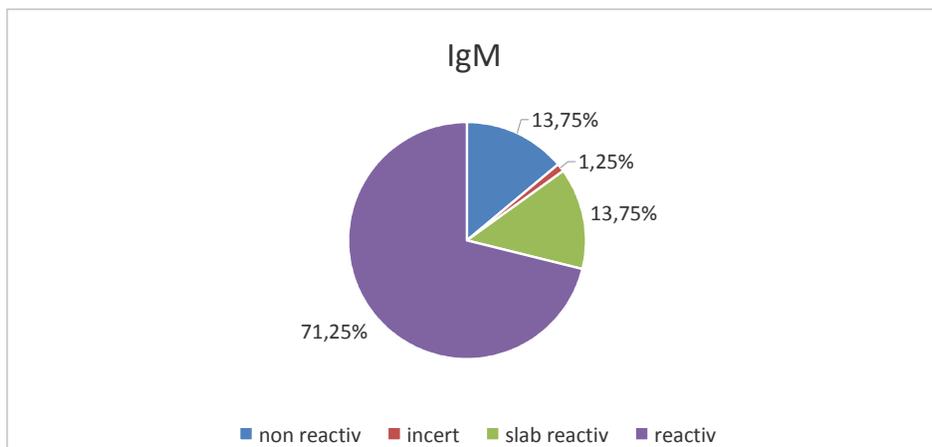


Fig. 9. Distribution of results for IgM antibodies against SARS CoV2 in the investigated persons

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Analyzing Fig. 9 and Fig 10 is found following the serum determinations, out of the 80 people detected reactive to at least one of the antibodies, 71.25% were reactive for IgM antibodies against SARS COV2 and 75% for IgG antibodies against SARS COV2, 13.75% of cases were weakly reactive for anti-SARS COV2 IgM antibodies and 21.25% for anti-SARS COV2 IgG antibodies. According to specialized data, IgM becomes detectable starting with the seventh day, reaches a maximum on the 14th day and disappears on the 21st day after infection, IgG appears on the 14th day, reaches a maximum on the 28th day and remains in the blood a longer term.

In our data it is found that 6 females had negative IgM antibody values, and for IgG weakly positive values and 3 females aged 41, 57, respectively 62 years had negative values for IgM

and IgG reactive values of 3.29; 3.3 and 11.44. (Fig. No.5). For the male staff, only two people, aged 29 and 36, presented negative values for IgM and weakly reactive values for IgG (1.37 and 1.64), (Fig. 6). All those people went throught the disease, being detected RT-PCR positive for SARS CoV2 in March, April and antibody values correspond to the ELISA determinations from September and October months.

In more severe infection, the longer of IgG antibodies persist. It should be noted that the female staff, a 40 years old person had a value of 4.57 for IgM and a value of 0.39 for IgG and a 52 years old person with weakly reactive IgM (1.37) and negative IgG (0.8). According to data from literature, for this situation, patients were in a period of decline, the patient begins recover and begins the production of IgG.
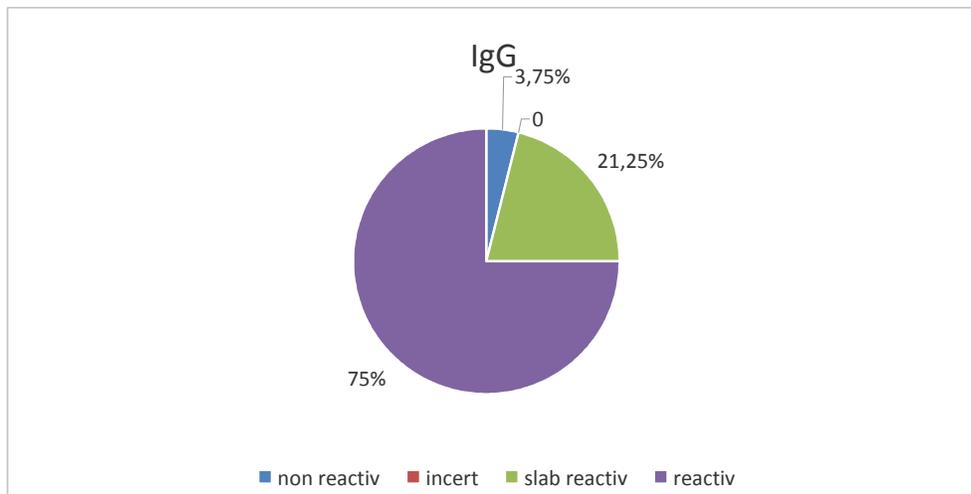


Fig. 10. Distribution of results for IgG antibodies against SARS CoV2 in the investigated persons
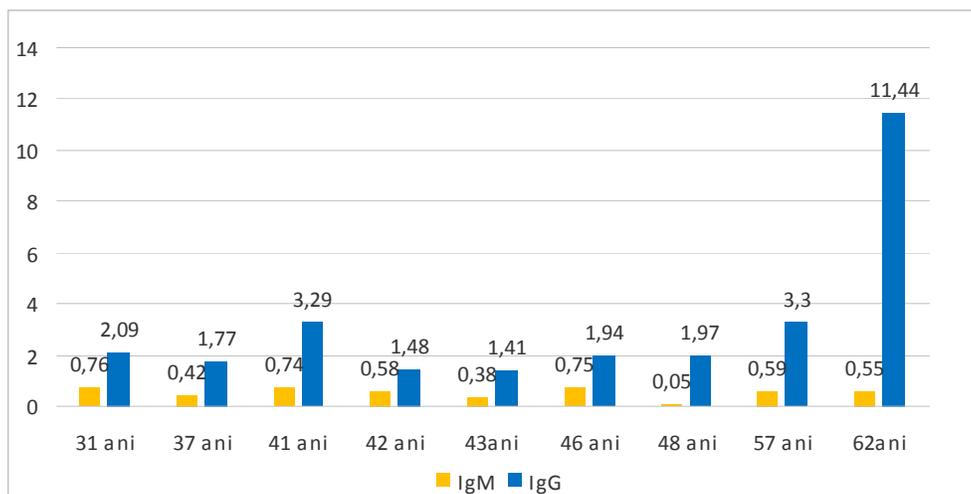


Fig. 11. Age of female staff with negative IgM and weakly reactive and reactive IgG
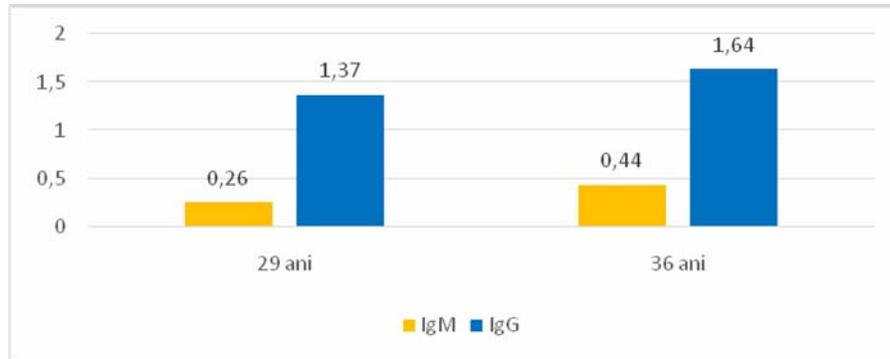
Fig. 12. Age of male staff with negative IgM and weakly reactive IgG

ELISA tests are used only for qualitative detections. A single test cannot be used for clinical diagnosis and treatment. The final diagnosis should include the clinical signs of the patients in conjunction with other tests. Patients may be IgM negative after the first week of infection or four weeks after infection with the new coronavirus. Also, patients with low immunity or other diseases that affect immune function, insufficient systemic organs and the use of drugs that suppress immune function can also lead to negative IgM and IgG results. Bacterical or fungal contamination of patient serum or reagents, cross-contamination between reagents may result in erroneous results.

## CONCLUSIONS

The data of this study showed that the results of serological tests were highly correlated with the results of RT-PCR tests.

This study showed that serological tests have a high accuracy and precision and can be successfully used for epidemiological surveillance by serological tests of medical staff, to assess the immune response due to natural infection inapparent or clinically apparent.

In the case of asymptomatic individuals, the final diagnosis of Covid-19 infection will be made by performing confirmatory tests for IgM and IgG and RT-PCR testing.

It is known if all infected patients have a protective immune response and how long this protective effect will last,which is why regular testing of patients identified with IgG is needed in the coming months.

The risk of diagnostic error in Covid-19 can be reduced by disseminating clear instructions for the collection, transport, handling, storage of biological samples and full compliance with all quality assurance procedures.

## ABSTRACT

The exact extent to which a person with SARS CoV2 infection can transmit the infection to other is uncertain. The potential for SARS-CoV-2 transmission begins before the onset of symptoms and decreases thereafter. Infected people are more likely to be contagious in the early stages of the disease, when the level of viral RNA in the upper respiratory tract is highest. The duration of viral RNA clearance is variable and may increase with disease severity. In some people, viral RNA can be detected in the respiratory tract a few months after the initial infection. However, detectable viral RNA does not always indicate the presence of the infectious virus and there appears to be a threshold below which the infectivity is unlikely.

Prevention and limitation measures are essential in both healthcare and the community. Medical personnel must apply additional precautions to protect themselves and prevent the transmission of the disease during medical care.

This paper aims to study the incidence of the IgM and IgG antibodies anti-SARS CoV2 in healthcare professionals. The determination of antibodies was made on request, both to see the immunization of people who went through the disease and to evaluate asymptomatic personnel (they came in direct contact with confirmed people).

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