Prevalence of SARS-CoV-2 infection in the workplace: results of a year of investigation in the Marche Nord companies

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Abstract. Background and aim: The world of work has been profoundly affected by the COVID-19 pandemic since workplace activities involving close contact with coworkers and customers can lead to transmission of SARS-CoV-2 and compromise continuity of operations of workplaces. The Covid-Lab of University of Urbino and the Confindustria Pesaro Urbino association signed an agreement to support the Marche Nord companies in the adoption of anti-contagion safety protocols. Methods: Antibodies detection was performed using a rapid immunochromatographic test. Total RNA from nasopharyngeal swab was subjected to a real-time RT-PCR multiplex for the detection of RdRp specific gene and E gene of the SARS-CoV-2, and the internal control (human RNase P). Results: Between May 2020 and Apr 2021, over 10,000 rapid serological tests had been carried out on workers of 35 companies and in 5% of cases IgG or IgM were found (519). All the 519 swabs gave a valid result (RNAse P Ct≤40) with 105 positive results (20%) for SARS-CoV-2 with a Ct value ≤45. Overall, only 1% of samples resulted positive for viral RNA (105/10000). Conclusions: The University of Urbino set up a rapid-response (within 24 h, generally <6 h) diagnostic centre for SARS-CoV-2 detection (Covid-Lab) allowing the companies to activate the optimal safety path to ensure the health and safety of workers in the workplace. Our observations during this first year of activity, highlight that in the workplace, the infection does not seem to spread if precautionary measures are followed and only 1% (1 worker out of 100) tested positive for the SARS-CoV-2 virus. (www.actabiomedica.it)

Key words: SARS-CoV-2 molecular tests, workplace, worker screening

Introduction

During the first months of the SARS-CoV-2 pandemic, the Covid-Lab set up at the University of Urbino was authorized (on 8th May 2020) to carry out molecular SARS-CoV-2 diagnostic tests for COVID-19 by the regional reference laboratory (Virology Unit, AOU Ospedali Riuniti, Ancona, Italy). Meanwhile the Confindustria Pesaro Urbino association (Confindustria is the main association representing manufacturing and service companies in Italy, with a voluntary membership of more than 150,000 companies of all sizes), as part of the procedures for implementation of the internal anti-contagion security protocols, signed an agreement with the Covid-Lab to support the Marche Nord companies about the containment of the
spread of the virus in the workplace and the health of its workers.

Serological tests are indirect tests, as they identify exposure to the SARS-CoV-2 by detecting any antibodies directed against the virus but are unable to confirm or not an infection in progress (1, 2). Although serological tests are very useful in research and epidemiological evaluation of viral circulation, they do not replace the search for viral RNA using the molecular technique (through a nasopharyngeal swab) that, for the time being, is the only conclusively diagnostic test (3). For this reason, the screening procedure developed involves a double step: firstly, a rapid test (performed in the workplace, always taken on a voluntary basis) that provides the response about the presence or absence of IgM and/or IgG or both antibodies, which is followed by a molecular swab to be administered only to antibody positive subjects, which is processed and analysed at the academic Covid-Lab on the same day as the rapid test. To ensure an optimal safety path, rapid tests are repeated periodically (every 2/4 weeks). The aim of this article was to present the results of a year of investigation on the prevalence of SARS-CoV-2 infection in the workplace, focusing our interest for the companies located in the northern area of the Marche region.

Methods

Immunochromatographic test

The detection of IgG and IgM antibodies was performed using a rapid immunochromatographic test CE-IVD certified (Diatheva srl, Cartoceto, Italy). The test provides the result in 15 min with the aid of a simple lancet device used to generate a blood drop at the fingertip and without the need for intravenous samples and any accessory equipment.

RNA extraction

Total RNAs from nasopharyngeal swabs were extracted using Total RNA Purification Kit (Norgen Biotek Corp., Thorold, ON Canada) starting from 250 µl of sample and following the Supplementary Protocol for Norgen's Saliva RNA Collection and Preservation Device.

Real-time RT-PCR multiplex assay

Three sets of primers and probes (4) were used to detect the envelope gene (E) (first line screening assay: E gene assay) and RNA-dependent RNA polymerase (RdRp) (confirmatory assay: RdRp gene assay) of the SARS-CoV-2, and the internal control (human RNase P) to evaluate RNA extraction and the presence of PCR inhibitors (COVID-19 PCR DIATHEVA Detection kit, Diatheva srl). The kit is a one-step real-time RT-PCR multiplex assay and is CE-IVD certified. Reaction and amplification conditions were performed according to the manufacturer's specifications. Briefly, 5 µL of extracted RNA were added to 15 µL of the reaction mixture and reactions were incubated at 48 °C for 38 min and 95 °C for 10 min followed by 50 cycles at 95 °C for 15 s and 58 °C for 30 s. PCR reactions were carried out in a 7500 real-time PCR system (Applied Biosystems, Thermo Fisher Scientific Inc.). Results were considered valid only when the cycle threshold (Ct) values of the RNase P reference gene were ≤ 40. The results were considered positive when the Ct values of RdRp target gene were ≤ 45, negative when > 45.

Subjects

The tests were conducted on a voluntary basis, the samples were received at the Covid-Lab anonymously and the participants had given consent to the treatment of their biological sample and data for research purposes.

Results

Between May 2020 and April 2021, over 10,000 rapid serological tests had been carried out on workers of 35 companies associated with Confindustria Pesaro Urbino, and in 5% (519) of cases IgG or IgM were found (Fig. 1A). Only for workers testing positive to antibodies it was necessary to perform the
nasopharyngeal swabs and the virus RNA analysis. All the 519 swabs gave a valid result (RNase P Ct ≤ 40, mean±SD 30.10±3.98) with 105 positive results (20%; Fig. 1B) for SARS-CoV-2 with a Ct value ≤ 45 (mean±SD 23.13±7.24). Overall, only 1% of samples resulted positive for viral RNA (105/10,000; Fig. 1C). In the months May–September 2020, the positivity rate was 0% (0/107 swabs), October–December 2020 19% (64/329) and in the months January–April 2021 49% (41/83), (Fig. 1D).

Based on AMCLI indications (5) we have evaluated the number of “very low positive” samples with RdRp Ct value ≥ 35. We observed 94 samples below (90%) and 11 (10%) above (Fig. 1E).

Due to the variety of swabs available on the market, for a subgroup of samples (n=436) we compared

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**Figure 1.** (A) Results of rapid serological tests and (B) nasopharyngeal swabs carried out on workers of 35 companies associated with Confindustria Pesaro Urbino. (C) Overall positivity rate. (D) Number of nasopharyngeal swabs processed in the Covid-Lab, set up at the University of Urbino, during the first year of activity and positivity rate for SARS-CoV-2 mRNA. (E) Ct value distribution (RdRp gene) for 105 SARS-CoV-2 positive samples.
nasopharyngeal swabs collected in two different viral transport medium [Zymo Research (1 mL fill) and Jiangsu Rongye Tecnology (3 mL fill)], (Fig. 2A). We noticed a shift in the Ct value of the RNAsP (Ct 6.1), for both positive (Ct 6.0) and negative (Ct 4.5) samples and samples collected in “3 mL swab” showed significantly higher Ct values (p<0.0001), (Fig. 2B). The spectrophotometric analysis confirmed that using “3 mL swab”, RNA samples had a lower concentration (mean±SD: 14±6 ng/µl vs 52±19 ng/µl, p<0.0001) and consequently as might be expected, a lower amount of RNA were used for subsequent RT-PCR test (mean±SD: 72±30 ng vs 259±95 ng, p<0.0001), (Fig. 2C). These results shed light that to avoid false negative results and problems due to low sensitivity with low viral load samples, the use of high-performance PCR kits is recommended.

Conclusions

The COVID-19 pandemic has driven demand for workplace surveillance tools. Workplace activities involving close contact with coworkers and customers can lead to transmission of SARS-CoV-2, the virus that causes COVID-19 (6).

The COVID-19 pandemic compromises continuity of operations of workplaces and the health and safety of workers.

During the current pandemic context, various collaborations have been built between industrial associations and COVID-19 reference laboratories (designated by the Ministry of Health) or laboratories that have obtained the diagnostic validation for SARS-CoV-2 infections by reference laboratories.
Among these, examples are those stipulated between Confindustria of various Italian cities with accredited territorial analysis laboratories and in particular that stipulated between Confindustria Pesaro Urbino and the diagnostic centre for Coronavirus (Covid-Lab) of the University of Urbino, which was established just following the COVID-19 health emergency. The collaboration made it possible to develop rapid two-step procedure that guaranteed the result within 24 hours (generally <6 h) allowing the company to activate the optimal safety path to ensure the health and safety of workers in the workplace, after the rapid case identification and isolation of infected subject. Furthermore, due to the repeated administration of rapid tests for the detection of IgM or IgG (approximately monthly), it was possible to contain the spread of the SARS-CoV-2 virus because it was constantly monitored, ensuring the continuity of the business activity, and avoiding costly closures for the company.

The findings in this report are subject to some limitations. First, only a part of member companies Confindustria Pesaro Urbino (35 of the 300 total) has voluntarily taken advantage of the agreement with the Covid-Lab. Secondly, the type of companies and the size of their employees is not uniform, so the risk of contagion is not the same among the various companies. Finally, it is taken in account that in the period in which the study was conducted the vaccination campaign was not yet fully operational so our results can be overestimated.

However, our observations during this first year of activity, highlight that in the workplace, the infection does not seem to spread if precautionary measures are followed and only 1% (1 worker out of 100) tested positive for the SARS-CoV-2 virus.

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