

***Title:**

Cellular and humoral immune responses after mRNA vaccination or SarS Cov 2 infection in a referenc laboratory in Greece

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***Abstarct: (250 Words)**

The purpose of this study was to evaluate cellular and humoral immunity after six month second shot mRNA vaccination or SARS-CoV-2 infection using the SARS COV 2 Interferon gamma release assay (Euroimmun) and IgG(ab) quantitative to spike protein using the Architect platform (Abbott). A total of 53 subjects and 11 individuals respectively after vaccination or mild illness and 12 healthy non vaccinated control group were included in the study. IgG spike levels were higher in high interferon gamma positive test concentrations individuals. Subjects after COVID 19 infection also have shown high levels of IgG when IGRA test was highly positive and with longer history of illness. mRNA vaccines demonstrate moderate to high levels of immunological responses in most of the individuals that correlate with IgG antibody levels. Although it is believed that IGRA-based tests should be performed within a few weeks following vaccination we demonstrated levels of T -cell response exhibiting ≥ 2000 mLU/mL correlated to ab levels ≥ 1000 aU/mL at 6 months after vaccination. Moreover, our 7 out of 11 convalescent individuals showed high T -cell responses correlating with high levels of antibodies. No significant difference was shown in levels of IGRA and ab between men and women. The developed tools to test immuno-triggering of mRNA vaccines and infection are of high importance in public health and they represent a useful tool in monitoring of immunity in non communicable chronic diseased patients.

Title: *Endorsement of high quality standards for the molecular detection of Sars Cov-2 RNA in a Reference Laboratory in Athens, Greece

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The objective of this study was to evaluate the efficiency of combined use of commercial RT-PCR methods for the molecular detection of SarsCov-2 RNA, in the presence of currently circulating Variants of Concern (VOCs) and how these variants may dynamically interfere with an analytical RealTime PCR result (Ct).

There is evidence that some VOCs (even on high viral load) may escape from exhibiting adequate fluorescent signal. Additional evidence shows that certain VOCs may present S gene dropout, leading to false negative results as regards the analysis of S gene, whereas certain PCR kit may be more susceptible to S gene dropout compared to others, when a certain VOC is analyzed.

For the purpose of this study, our lab proceeded in the statistical analysis of a sufficient number of External Quality Control samples regarding the schemes INSTAND & UK NEQAS External Quality Assurance of Coronaviruses Genome Detection (RNA).

The main result was that a given cycle cutoff (Ct) should be interpreted with care and in tandem with the current stage of the pandemic, as it may not always be directly connected with the viral load.

In parallel with this study, our laboratory tried to illustrate in diagrams the preference of the laboratories, in terms of most commonly used *kit manufacturer* and *genes targets*, as derived from the participations in the two well-known External Quality Assurance Schemes, while reflecting current laboratory practices and trends.

With a strong commitment to Quality, our laboratory's current Quality Assurance Practice follows the combined detection of five SarsCov-2 related genes (S/RdRP, E, N ORF1a/b) within the same limit of detection, which in turn is proved to lead to enhanced sensitivity while provides a safe protocol for Covid-19 diagnosis and quantification.

***Title:**

COVID- 19 second and third waves in Greece: experience of a molecular diagnosis reference laboratory

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The aim of this study was to depict the second and third waves in Greece and correlation to vaccination process and attitudes.

We tested about 120000 specimens by RT PCR, during March 2021 to March 2022. Table I panel I, shows the rates of vaccination and panel II rates of positive nasal samples to mRNA SARS COV 2 (five genes two Rt PCR kits). We tested a total of 118 152. Out of them 14 422 have been positive for SarS CoV 2 RNA. Fluctuation in positivity is shown in the table below panel II. We correlate also the rate of vaccination in the country with immunity and factors such as summer outdoors and physical activities and distancing. To conclude, a large spike in Greece COVID-19 cases occurred over the winter months of 2020–21 till the spring. The arrival of authorized vaccines in December 2020 helped bring new infection levels back down through the summer period that most of the specimens reflected simple preventive testing, and back the third wave late in December 2021 as the contagious delta variant began to circulate and become dominant and socializing during Christmas period in Greece is inevitable. Waning immunity and relaxation of public policies and infection prevention measures also played a role in the endemicity of the infection.