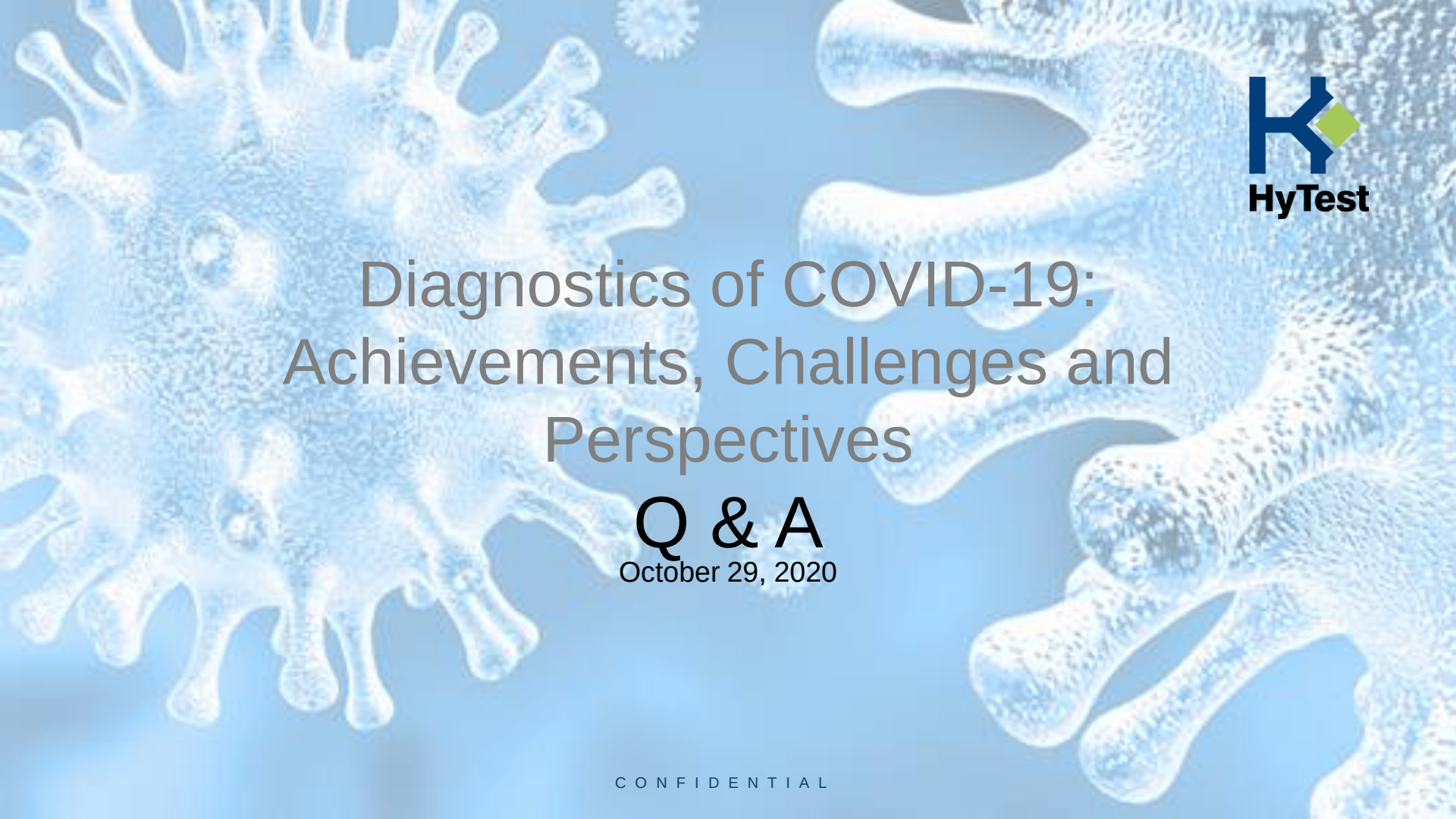

Diagnostics of COVID-19: Achievements, Challenges and Perspectives

Q & A

October 29, 2020

Questions & Answers

Q: What is the best antigen for developing a SARS-CoV-2 antigen ELISA?

A: Currently there are 6 FDA approved antigen tests that detect nucleoprotein and one antigen test that detects rbd of spike protein. Most of the published studies also describe assays detecting nucleoprotein. There are very few publications that describe development and performance of assays for detecting spike protein.

Many companies are currently working on development of assays for detection of Spike protein, so new data comparing the two proteins head-to-head will be available in the near future.

Although it is too early to determine whether one protein is better than the other, evidence supports the efficacy of nucleoprotein detection. Several publications indicated that detection of nucleoprotein in nasal swabs is diagnostic for early SARS-CoV-2 disease. In a study using an automated assay LUMIPULSE from Fujirebio strong correlation was observed between nucleoprotein levels and viral loads in hospitalized patients.

I would like to add that it is important not only to choose the right antigen, but also to choose antibodies with proper epitopes. First of all, coronavirus antigen tests must have very high detection specificity for accurate diagnosis. Therefore, antibodies that do not cross-react with other HCoV should be selected.

Secondly, Nucleoprotein and spike protein can be proteolytically cleaved in samples, and the remaining fragments can be undetectable by methods using antibodies specific to unstable regions. This can lead to a decrease in the sensitivity of the method. Consequently, the negative results using such antibodies cannot exclude SARS-CoV-2 virus infection.

Questions & Answers

Q: Is it possible to relate COVID-19 with troponins and what are the main relationships between them?

A: Now we know that SARS-CoV-2 may adversely affect the cardiovascular system. Systemic infection can trigger cardiac events. Numerous publications have identified cardiac troponin as an important prognostic biomarker for patients hospitalized with COVID-19. At present, there are limited demonstration of the virus in the myocardium and limited reports on definite diagnosis of myocarditis caused by SARS-CoV-2 in humans. As cardiac troponin (cTn) is a biomarker of disease severity and a powerful independent predictor of adverse outcomes, it may be quite useful for making decisions in the emergency department as well as for outpatient management. So, monitoring of cTn and other biomarkers such as the natriuretic peptides may provide additional utility.

Q: Are you recommend saliva samples as type of sars-cov-2 sample specimen and if yes, how significant compare to the nasopharyngeal swabs?

A: Well, nasopharyngeal swabs are considered the gold standard specimen type for SARS-CoV-2 testing. However, saliva is one among other non-nasopharyngeal swab collection methods including throat and nasal swabs for SARS-CoV-2 testing. Recent publications have shown good performance of saliva specimens for SARS-CoV-2 testing. The literature on the use of saliva for COVID-19 testing is rapidly evolving and expanding. To date, saliva appears to be an acceptable specimen, though it is generally less sensitive than nasopharyngeal swabs. So, it can be concluded that saliva is a reliable noninvasive specimen type to consider for use in the ambulatory, non-hospitalized setting.

Questions & Answers

Q: Which SARS-CoV-2 protein (Spike or nucleoprotein) show the best performance for IgG and IgM detection?
A: Serological tests typically detect antibodies against spike protein or nucleoprotein since these are the most immunogenic proteins of SARS-CoV-2. To date, there is no consensus which antigen is better for detection of SARS CoV-2 antibodies.

It was suggested that detection of antibodies against spike protein could provide a better indication of neutralizing antibody titer. However, current evidence is insufficient to prove that assays which employ S1 subunit or RBD region show a greater correlation to antibody neutralization activity than assays using nucleoprotein. In addition, according to published data serological assays exhibit excellent agreement regardless of the target antigen used.

Secondly, one major concern regarding the implementation of serological testing into clinical practice is the potential for cross-reactivity, especially given that over 90% of adults are estimated to have antibodies against other commonly circulating coronaviruses. It was suggested that assays using nucleoprotein protein might be more to false positive results since nucleoprotein has several conserved regions with high sequence homology to other HCoVs such as common cold viruses HCoV-229E, -NL63, -OC43 and -HKU1. However, it was demonstrated that immunoassays using nucleoprotein (for example, Abbott and Roche assays) demonstrate excellent specificities.

Thirdly, for detection of recent infection, it might be preferable to use an antigen for which seroconversion occurs faster. However, it is not clear if seroconversion occurs faster for one of the proteins. (Answer continues, see next slide)

Questions & Answers

(Answer continues, see previous slide)

So, information that might impact serologic recommendations is rapidly evolving. Several key issues, including: the kinetics of antibody response, the length of antibody persistence, the ability of antibodies to protect from repeat infection, and the correlation of binding antibody titers to neutralization ability are yet to be determined.

Therefore, we can expect that we will be get an answer to this question in the nearest future. It is possible that in future different antigens will be used for different purposes. For example, spike protein will be used to confirm response to vaccination, whereas nucleoprotein will be used for detection of recent or prior viral infection.

Q: Solutions to cross-reaction in antigen detection and antibody serology tests. Improving reagent specificity
A: SARS-CoV-2 is one of 7 of seven coronaviruses identified so far that are known to infect humans. In case of antigen tests, a critical issue is evaluation of cross-reactivity of antibodies to the selected target from other coronaviruses. Antibodies with no or very low cross-reactivity should be utilized in antigen tests.

In case of serological tests, a proper validation is needed. In this analysis several sources of specimens should be included. Symptomatic patients who have tested negative for COVID-19 by molecular assays and specimens acquired prior to the COVID-19 outbreak, specimens from patients with confirmed endemic coronaviruses should be included. Additionally, specimens with heterophile antibodies and rheumatoid factor should be analyzed to determine the false-positive rate.

Questions & Answers

Q: SARS-CoV-2 positive patient samples (Serum/plasma/whole blood) handling safety, recovered vs currently sick. A: There are recommendations published by WHO, IFCC, CDC, and other local organizations for handling and testing clinical specimens during the COVID-19 pandemic. The provided recommendations are slightly different; therefore, one should follow local guidelines. It is recommended that clinical laboratories perform their own risk assessment for handling biological specimens during the COVID-19 pandemic.

According to CDC: Routine diagnostic testing of specimens that are suspected or confirmed for SARS-CoV-2 can be handled in a BSL-2 laboratory using Standard Precautions. IFCC provided a set of recommendations on biosafety measures for clinical chemistry laboratories that operate at biosafety levels 1 and 2. According to these recommendations during the COVID-19 pandemic, all specimens collected for in vitro diagnostic testing should be considered potentially infectious with SARS-CoV-2.

Laboratory professionals must wear PPE (i.e. masks and gloves, laboratory coat or gown, and eye protection) at all times. Conventional laboratory coats can be used when managing usual biological materials, where the threat of infection is reasonably lower (blood and urine).

Regarding facemasks, the majority of the official documents recommend that laboratory professionals should wear preferably an N95 mask while engaged in all types of aerosol-generating procedures related to non-centrifuged samples (i.e. manually opening specimen tubes, pipetting, vortexing, aliquoting, shaking, centrifuging, extracting) carried out on samples potentially contaminated with SARS-CoV-2. For enhanced safety reasons, this equipment may also be used when BSL1 or BSL2 cabinets are used, even if the cabinet provides protection and shield. (Answer continues, see next slide)

Questions & Answers

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For routine testing of blood and urine, laboratories should use automated instruments and analyzers with closed preanalytical robotics, where possible.

When manual laboratory techniques are used for processing specimens there is a risk of generating aerosols. Therefore, according to IFCC guidelines, manual processing such as opening tubes, pipetting, aliquoting, diluting, vortexing and extracting specimens should be handled in a BSL 2 cabinet to avoid contamination during performance of aerosol-producing activities.

Centrifugation of specimens should be performed using sealed rotors or sample cups which are loaded and unloaded in BCL2 cabinet.

Laboratory staff should decontaminate working surfaces with standard disinfectants approved for SARS-CoV-2 infection.


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