SARS-CoV-2 antigen tests: safety for less than 5 cents per test

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Saliva or swab tests are essential for pathogen detection in respiratory diseases and will be increasingly used for immunity detection after Covid vaccination in the future. In this context, the potentially high infectivity of swab or saliva samples often causes user hazards and associated regulatory hurdles. However, safe design using the SafetyTector™ S virus-inactivating buffer for saliva and swab tests, like SARS-CoV-2 antigen rapid tests, is easily achievable. User protection is not expensive at 2 to 5 ¢ per rapid test, depending on test volume and production lots. Savings on in-house production of diluent further reduce costs.

The CoViD-19 pandemic highlighted the importance of good diagnostics in controlling infectious diseases to the general public. In addition to early established diagnostics using nucleic acid amplifying tests (NAT), such as the now widely known polymerase chain reaction (PCR), SARS-CoV-2 antigen tests have gained importance in recent months and can also be used as point-of-care (POC) diagnostics. Here, a distinction must be made between tests for professional use and lay tests, such as those also performed by schoolchildren. The latter are often based on swabs of the anterior oral or nasal mucosa or on saliva samples for direct pathogen detection. According to recent findings, saliva in particular allows for a higher sensitivity in pathogen detection than swabs of the nasopharynx or nasal swabs taken by patients themselves[1].

In addition, saliva has also been shown to be suitable as a sample matrix for the detection of SARS-CoV-2 specific IgG antibodies in serological test formats[2]. The so-called "mucosal immunity"mediated by IgA antibodies is thus also easy to measure. This helps to prevent immune individuals from becoming infectious. Saliva samples are also a good sample matrix for the detection of antibodies against other pathogens, as the results correlate well with the results of serological serum tests[3]. The ease and convenience of sample collection for the patient is a major advantage of saliva-based testing systems, especially for lay use.

In the context of the SARS-CoV-2 pandemic, however, a disadvantage of these test systems becomes apparent: In general, patient samples must always be considered potentially infectious. This problem is exacerbated in times of pandemic spread of a respiratory virus, where infected individuals are already pre-symptomatically infectious and asymptomatic courses are common. The WHO recommends that handling of specimens to detect SARS-CoV-2 takes place in a biological safety cabinet prior to inactivation[4]. However, these tests are performed in schools, kindergartens, nursing homes, and workplaces without adequate safeguards, let alone safety cabinets.

This poses a problem especially for manufacturers of in vitro diagnostics. Mitigating the risk to users is a mandatory part of the validation of a newly developed diagnostic. ISO 14971, "Application of risk management to medical devices"[5] which governs the implementation of ISO 13485, states that the medical device should be designed and manufactured to be inherently safe (safe design). The In Vitro Diagnostics Regulation[6], which is mandatory in the European Union from May 26, 2022, also requires manufacturers to "eliminate or reduce risks as far as possible through safe design and manufacture". Only if this is not possible according to the state of the art, protective measures or safety information are an acceptable means of risk reduction (see Fig. 1).

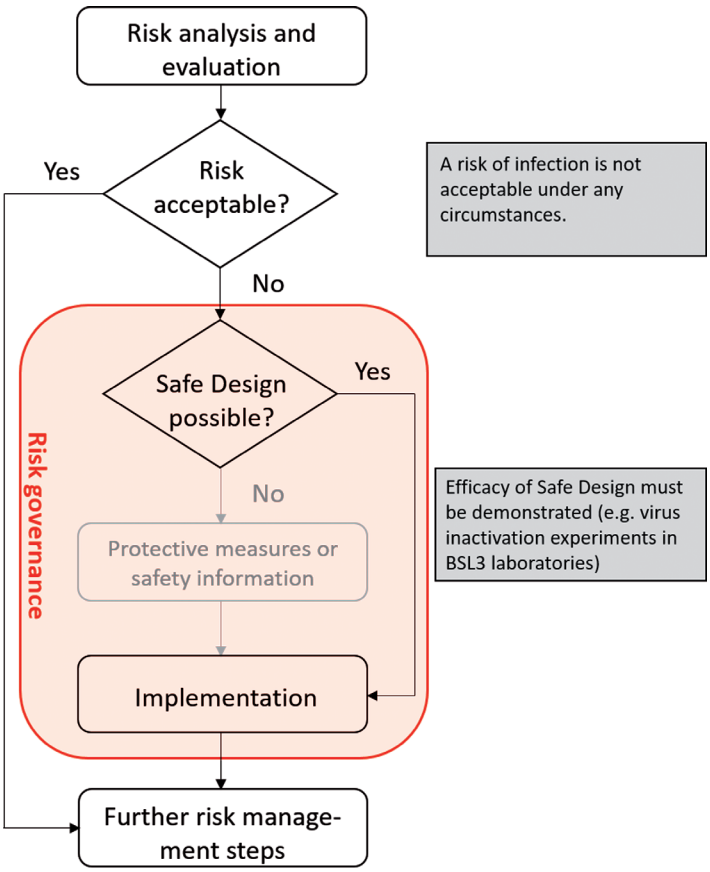

Fig. 1: Prioritization of risk control options according to ISO 14971. If feasible, medical devices shall be designed to be inherently safe (safe design). Only if this is not possible, protective measures or safety information are acceptable as risk control measures. For user protection, virus-inactivating buffers are therefore preferable to protective measures (e.g. gloves, protective goggles, tear-proof collection bags) or safety information (e.g. in the package insert).

The extraction buffers used in PCR diagnostics for respiratory samples are generally virus-inactivating and thus meet the requirements of safe design. Antigen tests, on the other hand, often operate with milder buffers that will not necessarily inactivate SARS-CoV-2. As a result, many diluents currently used in rapid tests do not have a sufficient inactivating effect[7]. Excess specimen and test cassettes remain infectious after testing and pose a health risk. Particularly at sites where large-scale testing is being conducted in a pandemic, this is a completely unnecessary hazardous situation that can be avoided by using virus-inactivating dilution buffers. Unfortunately, however, it is precisely this element of the safe design approach that has been neglected or insufficiently demonstrated in the development of many antigen tests on the market. Even when a virus-inactivating effect is claimed, it has only in exceedingly rare cases been confirmed by meaningful experiments with real virus isolates in biosafety level 3 (BSL-3) laboratories. However, without sufficient data, user protection cannot be claimed or guaranteed. Without evidence, an auditor cannot make a positive decision to place a diagnostic on the market.

The solution: SafetyTector™ S

To solve the problem of virus inactivation and to make safe dilution buffers available to all market players without major investment and time, CANDOR has developed SafetyTector™ S. SafetyTector™ S is a ready-to-use virus-inactivating dilution and extraction buffer for saliva samples and nasal and pharyngeal swabs. At the same time, SafetyTector™ S is not a hazardous substance. Moreover, the virus-inactivating effect is achieved without the use of environmentally harmful virucidal components, such as the commonly used endocrine disruptor Triton X-100[8]. Triton X-100 must not be used any longer for products in many international markets.

To be able to guarantee the safety of users, the virus-inactivating effect must be verified experimentally. In tests with infectious isolates of SARS-CoV-2, of influenza A virus and of herpes simplex virus 1 diluted in saliva, which were performed in a BSL3 laboratory at the Ulm University Medical Center by the group of Prof. Dr. Jan Münch, no residual infectivity could be detected after only 1-minute incubation with SafetyTector™ S (Fig. 2). Comparable results were obtained in tests with other human pathogens - measles virus, herpes simplex virus 2, Zika virus - and with the SARS-CoV-2 variants B.1.1.7 (“British variant“) and B.1.351 (“South African variant“).

SafetyTector™ S thus has a broad anti-viral effect and reduces the risk to users during the analysis of saliva and swab samples from potentially infectious patients.

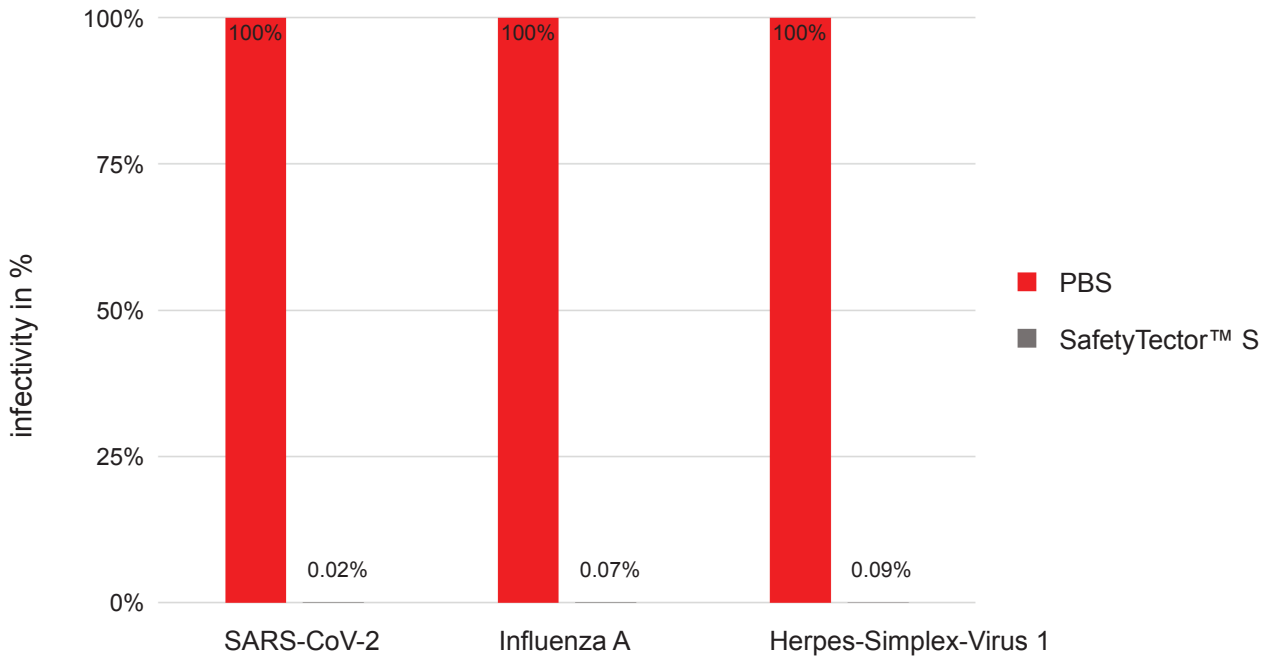

Fig. 2: SafetyTector™ S has broad antiviral activity. Saliva samples spiked with SARS-CoV-2, influenza A virus or herpes simplex virus 1 (HSV-1) were mixed 1:4 with PBS or CANDOR's SafetyTector™ S and incubated for 1 minute at room temperature (n = 3). After incubation, samples were titrated and added to Vero E6 (SARS-Cov-2 and HSV-1) or MDCK (influenza A virus) cells. After four to seven days, infectivity was quantified by the Reed-Muench method. For all three viruses tested, infectivity was no longer detectable after incubation with SafetyTector™ S using this method.

Analytical Performance

In addition to safety for the user, a strong analytical performance is essential in the development and validation of a diagnostic kit. Selection of the right diluent is critical for the performance. One challenge in analyzing potentially infectious samples is to generate a virus-inactivating effect that does not interfere with antigen-antibody interaction, leading to false results. CANDOR has achieved this balance in the development of SafetyTector™ S. Analytical performance was investigated by replacing the respective supplied dilution and extraction buffer with SafetyTector™ S in commercially available rapid antigen tests listed by the German Federal Institute for Drugs and Medical Devices (BfArM). In these different rapid tests, the detection limit remained unchanged low when SafetyTector™ S was used and no false positive results were obtained (Fig. 3 A - D). Although the rapid tests were not developed with SafetyTector™ S, it is on a par with the original diluents. By directly incorporating the SafetyTector™ S into the development and optimization of a saliva or swab-based immunoassay, the problem of user safety can thus be solved without compromising analytical performance.

In addition, due to its composition, SafetyTector™ S is able to improve the often-problematic texture and viscosity of many saliva samples - and thus their flow properties - which can be an advantage in fluidics-based immunoassays, such as lateral flow assays.

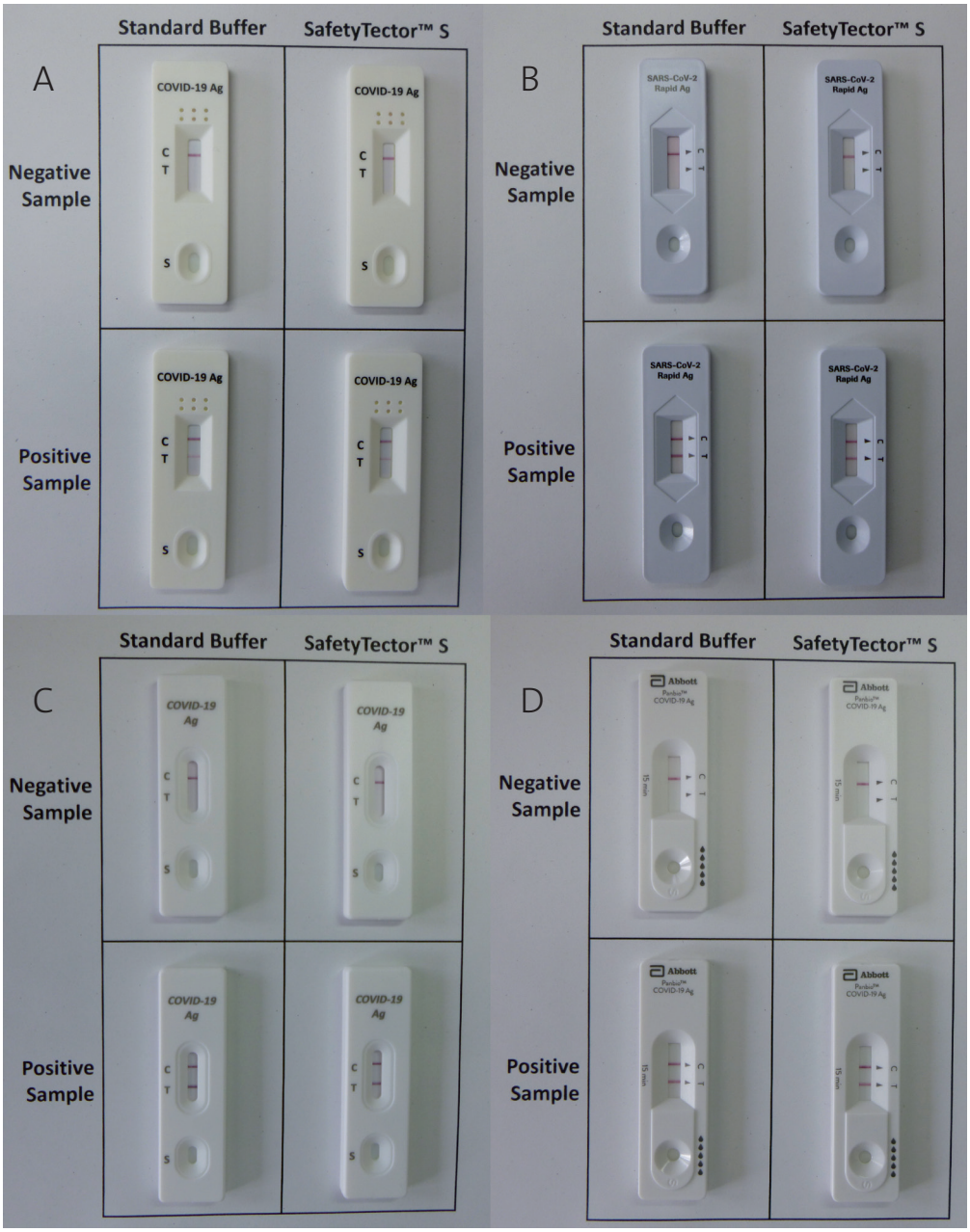

Fig. 3: SafetyTector™ S is compatible with rapid antigen tests from various manufacturers. Saliva samples from healthy donors were run either untreated (Negative Sample) or after addition of SARS-CoV-2 antigens (Positive Sample) according to the package insert using either the supplied dilution buffer (Standard Buffer) or SafetyTector™ S.
Manufacturers: A) DRG Instruments; B) Roche; C) BTNX; D) Abbott

The costs are negligible: When produced on a commercial scale, they are typically between 2 and 5 ¢ per test when calculated on a per-test basis for common volumes of diluent. In addition, costs for the replaced diluents are saved, so that the actual additional costs are even lower.

SafetyTector™ S - the new state of the art

SafetyTector™ S developed by CANDOR is a dilution and extraction buffer for immunoassays that can inactivate viruses contained in saliva or swab samples and thus significantly improve the user safety of in vitro diagnostics. The analytical performance of the diagnostic remains unchanged at a high level. SafetyTector™ S thus represents a new state of the art for immunoassays. The use of SafetyTector™ S saves resources and time in development, risk management and validation of diagnostic immunoassays. Tests based on this technology can also be safely performed by non-professionals in community settings such as schools. Also, proper disposal of used test kits is no longer a challenge for users.

CANDOR's EN ISO 13485-certified production facility in Wangen/Germany guarantees the consistently high quality of the SafetyTector™ S and enables batch sizes of up to 1000 L.
Test samples are available for developers and manufacturers of in vitro diagnostics on request.

References:

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4: World Health Organization (2020) Laboratory biosafety guidance related to coronavirus disease (COVID-19)
5: EN ISO 14971:2019, Annex A, Section A.2.7.1 Risk Control Option Analysis
6: Regulation (EU) 2017/746, Annex I, Chapter I, paragraph 4 (In-vitro-Diagnostics Regulation)
7: Public Health England (2020) COVID-19: PHE laboratory assessments of inactivation methods
8: List of substances included in Annex XIV of REACH („Authorisation List"), Entry 4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated (https://www.echa.europa.eu/web/guest/authorisation-list/-/dislist/details/0b0236e1807df80d)

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Supported by:
Federal Ministry for Economic Affairs and Energy
on the basis of a decision by the German Bundestag

V-WSTE-05-2021

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