INSTRUCTION MANUAL

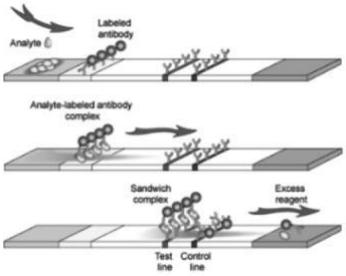
Read the instructions carefully before the test is performed. Please follow the instructions and do not change the process. Thanks to strictly following the instructions, you will avoid inaccurate results and reach the optimal result of the product Saligen.

Name of the product
Saligen

Intended use
Saligen is a diagnostic medical product in vitro based on the principle of immunochromatography assay (ICA), that qualitatively detects SARS-CoV-2 antigens in the human saliva. This test is used for the detection of SARS-CoV-2 antigens in people suspected from COV-ID-19 disease. This product is designed for professional purposes or other according to the decision of each given country.

Summary and explanation of the disease
COVID-19 is a respiratory disease caused by the new type of coronavirus (SARS-CoV-2), first identified in Wuhan, China, in December 2019. Common symptoms usually include respiratory symptoms, fever, cough, shortness of breath, loss of smell and taste. Severe symptoms include pneumonia, severe acute respiratory syndrome, kidney failure and even death. Coronavirus is a group of viruses causing symptoms variable from mild (e.g. cold) to severe.

Principle of the test
Saligen detects N protein. It uses COVID-19 antibodies labelled by the small particles of gold, which are tagged to the nitrocellulose membrane of the testing cassette near the hole for the sample. The sample is absorbed from the hole into the reagent area due to the effect of the capillary action. After the sample meets the tagged gold-labelled antibody, the antibodies are released from the membrane and flow further through the whole reagent area of the testing cassette.



If the sample contains SARS-CoV-2 antigens, the antigens then bind to the gold-labelled antibodies and form antigen-antibody complexes. These complexes flow through the nitrocellulose membrane to the next test line (T), where are caught by other anti-COVID-19 anti-bodies and form the sandwich complex, which causes the colourful strip. The complex antigen-antibody does not form in case there are no SARS-CoV-2 antigens in the sample and therefore a colourful strip does not appear in the testing “T” line. There has to be the sign of colourful strip in the control area (C) with or without the presence of SARS-CoV-2 antigens in the sample. If there is no colourful strip in the control area (C), the test is invalid.

Test values
Sensitivity: 95%
Specificity: 99%
Number of persons tested: 140

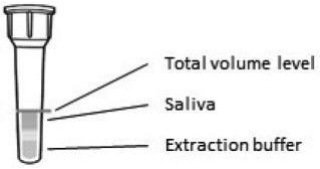
Contents of the kit
1) Testing card
2) Extraction buffer tube
3) Filter cap
4) Mouthpiece for easier saliva collection

Required materials, that are not part of the kit
• Chronometer or stopwatch

Storing and the kit stability
• Testing kit Saligen should be stored at temperature 2 - 30°C in a dry place. After following the instructions of storing and manipulating, the testing cards with the reagents are stable until the expiration date written on the label of the kit.
• Use the testing cards immediately after the opening of the sack.

Sample collection
Do not eat, smoke, chew or drink anything except water minimally 30 minutes before the test. **Recommendation: Cough few times before the test to reach more accurate result. Collect bigger amount of the saliva in the mouth and move it front to back several times.**

Saliva samples
• Tested person collects the saliva in the mouth on the top of the tongue for 30 seconds (approx. 0.5 mL); see the illustration below.
• Spit the collected saliva right into the extraction buffer tube for the immediate use. You can use mouthpiece for easier saliva collection. Total volume in the tube should double after the addition of saliva **(do not count the foam, only the liquid part).**



• Do not use stored samples. Long-term storing can lead to the lower signal.
• Prevent sample from the frost.

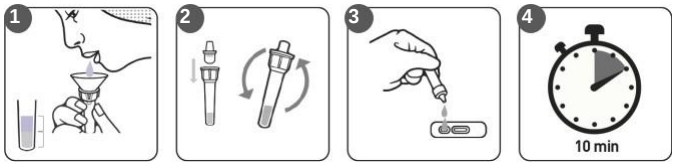
Warnings and precautions
• This product is designed for in vitro diagnostic use.
• This product is designed for single use only.
• This product is designed for professional purposes or other according to the decision of each given country.
• This product is designed for POCT using human saliva.
• Follow the instructions to reach the accurate result.
• Do not use after the expiration date or in case of damaged kit.
• Do not use other reagents, which are not included and do not mix components of different batches.
• This reagent can be used at room temperature (15 - 30°C). Let the samples, that were collected at lower temperature, heat to room temperature before use.
• Take out the testing card from the cover and use it as soon as possible and prevent long-term exposure to air, which affects the results of the test.
• Follow the laboratory test procedures during infectious diseases.
• Waste after the use of the product should be disposed as an infectious material and should not be disposed in a standard way.
• Proper instructions should be set to reach the safety of infectious reagents and materials.
• Use the gloves during the use of samples and reagents.
• Do not put reagents and samples into the mouth.
• Do not smoke, eat, drink, use cosmetic or contact lenses during the use of the product.
• Spilled samples and reagents should be cleaned by disinfectants.
• Disinfect and dispose all samples, reagents and contaminated materials according to the current legislation.

Preparation for use
The chemicals should be placed at the room temperature for 20 to 30 minutes. Do not use the samples stored for longer time.

Saligen

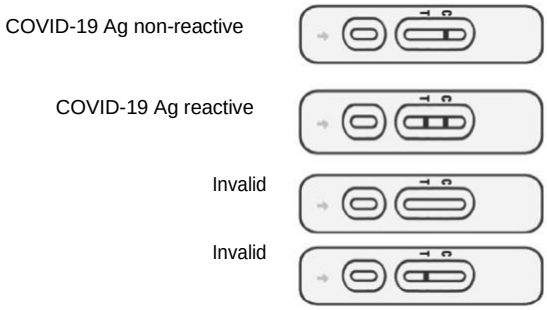
Procedure of evaluation of the testing card

1. Collect the sample according to the instructions – see the sections "Sample collection" and "Saliva samples".
2. Put the cover with the filter on the tube and tighten it. Mix the con-tents by turning the tube upside down and up (10x). Open the sack with the testing card right before the use. If the card is not used straight after the opening, the result could be inaccurate.
3. Open the sack with the testing card and place the testing card on a flat surface. Put few drops of mixture of extraction buffer and saliva into the hole of the testing card. The hole should be filled up completely. Make sure you put there 3 - 4 drops.
4. Read the result after 10 - 20 minutes.



Reading the result on the testing card after more than 20 minutes can lead to the inaccurate results.

Interpretation of results



Three different results can appear while using the testing card:

1. The result is valid and "non-reactive" if a red strip shows in the testing part at the "C" level, which means there are no SARS-CoV-2 antigens in the sample and the sample is **NEGATIVE**.
2. The result is valid and "reactive" if a second red strip shows in the testing part at the "T" level, which means there are SARS-CoV-2 antigens in the sample and the sample is **POSITIVE**.
3. The result is invalid if there is no red strip or if a red strip shows only at the "T" level. In this case the result cannot be used because the test did not work properly. See the detail in the section "Internal control". The sample is **INVALID**.

The tested person should not draw any conclusions about the health impact of the results obtained without consulting their doctor. The patient can only change the treatment if he has been properly trained in this regard.

Internal Control

There is an integrated inner control component in Saligen testing card. Red strip shown at the "C" level is designed as an inner control strip. Significant red strip means there has been adequate flow and the testing card has worked. If there is no control strip within 10 minutes, it is considered as a mistake and it is recommended to repeat the test again with the same sample and new testing card. Contact the producer or the distributor if the problem continues.

External Controls

- Positive and negative controls can be used with Saligen testing kit. These controls provide other material for the quality control, which evaluate the right reaction of the reagents. Positive controls have to lead to "reactive" results and negative controls to "non-reactive" results.
- It is recommended to start the new control for every new batch.
- Contact the producer or the distributor if the testing kit does not work properly and do not tell the patients the test results.

Test limits

- Do not consider the results of Saligen testing kit as absolute and single way of detecting the infected person. The infection should be confirmed by the specialist evaluating other experimental results, symptoms and other clinical information.
- This kit detects SARS-CoV and SARS-CoV-2 regardless of their viability. This kit cannot distinguish between SARS-CoV and SARS-CoV-2.
- Low antigen levels at the beginning of the disease can lead to the non-reactive result.
- Considering the limited analytical methods, the non-reactive results cannot fully eliminate the possibility of the disease.
- This product can detect SARS-CoV and SARS-CoV-2 antigens qualitatively in the human saliva and can not specify the amount of the antigens in the sample.
- In silico analysis of UK (B.1.1.7) and South African (B.1.351) mutations of SARS-CoV-2 did not find any direct risks considering a diagnostic efficiency of Saligen kit.

Efficiency characteristics

Limit of detection (LoD)

LoD has been set by the limit dilution of inactivated SARS-CoV-2 (ZeptoMetrix, #0810587CFHI) using two separated methods. Inactivated virus has been injected into the extraction buffer processed by non-reactive saliva sample concentration $TCID^{50}$ 1.15 x 106/mL. Every sample has been serially ten times diluted and LoD with 100% (3/3) reactive velocity has been set preliminary by three conducted tests. Four concentrations under the lowest concentration in 20 replications were tested before the exam to confirm LoD study and for Saligen LoD was used concentration showing 100 % (20/20) reactive results.

- Saliva LoD: 5.62 x 102 $TCID^{50}$/mL Cross reactivity/microbial interferation.

It has been confirmed that viruses/bacteriae listed below do not have cross reactivity and do not interfere with Saligen kit.

- Viruses (105 TCID50/mL): Adenovirus type 1, Adenovirus type 7, Coronavirus 229E, Coronavirus NL63, Coronavirus OC43, MERS-CoV, Cytomegalovirus, Influenza A H3N2, Influenza A H1N1, Influenza B, Enterovirus type 71, Parainfluenza type 1, Parainfluenza type 2, Parainfluenza type 3, Parainfluenza type 4A, measles virus, human metaphysis virus, type RSV, type B, influenza virus
- Bacteriae (106 CFU/mL): B. pertussis, E. coli, H. influenzae, M. catarrhalis, C. pneumoniae, L. pneumophila, M. pneumoniae, M. tuberculosis, N. meningitidis, P. aeruginosa, S. epidermidis, S. pneumoniae, S. pyogenes, S. salivarius and S. aureus

Endogenous interference

Possible interfering substances listed below does not react with Saligen.

- Mucin (4 mg/mL), human blood (2%), 4-acetamidophenol (10 mg/ mL), acetylsalicylic acid (20 mg/mL), chlorpheniramine (5 mg/mL), diphenhydramine (5 mg/mL), guaiacol glyceryl ether (20 mg/mL), oxymetazoline (0.05 mg/mL), phenyl (1 mg/mL), fenoxofrin (µg/ mL), mupirocinum (10 mg/mL), fluticasone propionate (5%).

LOT Number of batch	IVD Medical product in vitro	Follow the instruction manual
Sufficient amount for n tests	Store at 2-30°C	Do not reuse
Expiration Date	Producer	Warning
Compliance with European rules		

Bakter Medical s.r.o.
Chaloupky 171/33, Komín, 624 00 Brno
IČO: 09418806 DIČ: CZ09418806
Tel: +420 725 395 600
www.saligen.com

Issued on : June 28th, 2021
Version: 2.0

Saligen