

Guide for Trainers

Validation and Workflows in SARS-CoV-2 Testing Centers



Version 3.0

Last updated on: 15 /08/ 2021



Contents

1 Foreword	5
1.1 Executive Summary	6
2 Introduction	9
2.1 Background	9
3 Overview	11
4 Practical information	13
4.1 Facilities and equipment	13
4.2 Target audience	14
4.3 Course Language	14
5 Presentations	15
5.1 Introduction	15
5.2 Module II - Types of SARS-CoV-2 Tests	15
5.3 Module III - Validation of test kits	31
5.4 Module IV - Quality Management	45
5.5 Module V - Workflow	57
6 Practical trainings	71
6.1 Introduction to Practical training	71
6.2 Donning & Doffing	72
6.3 Sample taking & rapid test	72
6.4 Standard Operation Procedures	73
6.5 Root Cause Analysis	74
6.6 Design test site	74
6.7 Setup test site	74
6.8 Simulate test site	76
Appendix I - list materials	79
6.9 Material, supplies and kits for demonstration and practical . . .	79
7 Frequently Asked Questions	81

7.1	Definitions	81
7.2	Algorithm	82
7.3	Testing	83

Chapter 1

Foreword

This guide for teachers is part of the EU project 73 action plan for the COVID19 emergency response activities in Lebanon. A major part of action plan has to be delivered by trainees of first responders. The guide forms a handbook for the trainers to deliver the training on COVID19 emergency response, in particular for setting-up and operating Rapid Testing Facilities.



Trainers specification:

To train the content of the COVID19 modules, trainers should be selected. Good teaching skills is key in transferring knowledge. As the content is taught to first responders, it is key to have a confident and hands-on teacher delivering the training.

Minimum qualifications:

- Good training skills are key in transferring knowledge.
- Professionally connected to community health service provider
- Computer literate

Additional relevant experiences:

- Background in C, B and/or RN
- Experience in clinical diagnostic settings.

Acknowledgements

Material for the modules was contributed by the Amsterdam Municipality Health Services (GGD-Amsterdam), the COVID19 testing center in Kranenburg, Germany and various staff-members at the RIVM.

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1.1 Executive Summary

This guide for trainers contains an introduction of the curriculum, guidance on the curriculum structure and objectives and an overview of the modules and individual topics therein. The COVID19 curriculum covers the following modules:

Module I: Introduction

Module II: Types of Rapid Tests

Module III: Validation of Test Kits

1.1. EXECUTIVE SUMMARY

7

Module IV: Quality Management in Testing Center

Module V: Set-up and Workflow in Testing Center

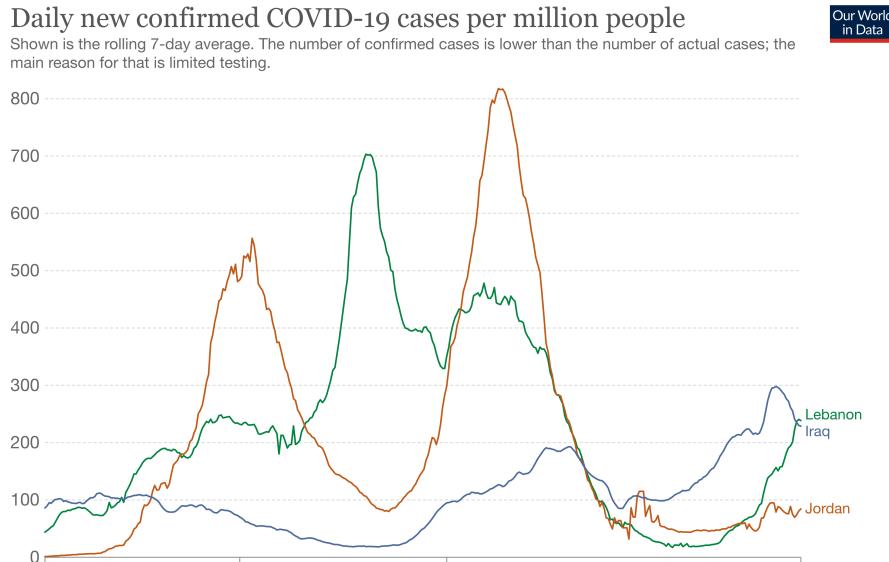
Module VI: Practical training

Chapter 2

Introduction

2.1 Background

In Lebanon, the Coronavirus disease (COVID-19) pandemic has disrupted the lives of inhabitants and forced the country to devise public health policies to reduce the pace of transmission. The new registered cases in the region, as shown in the graph, show an erratic pattern. This pattern will likely continue as long as there is no control over the spread of the virus.



Source: Johns Hopkins University CSSE COVID-19 Data

CC BY

The graph was updated on 15/08/2021. Source: Our world in data

The socio-political tensions and the large refugee populations have increased the disease burden of COVID-19. The support of first responders are particularly necessary to implement effective disease control measures to reduce SARS-CoV-2 persistence and transmission. Measures that are designed to reduce aerosol transmission within the population must be implemented, which include universal masking and regular, widespread testing to identify and isolate infected symptomatic and asymptomatic individuals.

Testing of SARS-CoV-2 is central to COVID-19 management and relies heavily on rt-PCR technology. For public health measures, another approach is needed in which the focus is to determine within minutes whether a person is infectious, instead of determining if a person is carrier of the virus (nucleic acid).

For rapid and cheap SARS-CoV-2 testing, a large number of rapid tests are offered on the global market. The reliability of the test should undergo thorough examination before they are allowed for use. After validation, test kits should be monitored continuously.

The training explains the different types of rapid tests. The participants will learn to setup and perform a validation of a rapid test. Secondly, the participants will learn to conduct continues quality monitoring. Finally, the participants will learn to setup save and efficient workflows in testing-centres.

This guide for trainers describes the material and activities that can be applied to achieve the optimal outcomes when transferring knowledge and practices on controlling COVID-19.

Chapter 3

Overview

Summary overview of his guide.

Overview of content

Length Practical information	Content	Duration
	Facilities, equipment, target audience	N/A
Presentations	<ul style="list-style-type: none">• Module II - Types of SARS-CoV-2 Tests• Quiz questions• FAQ• Module II - Types of SARS-CoV-2 Tests• Quiz questions• FAQ• Module III - Validation of test kits• Quiz questions• FAQ• Module IV - Quality Management• Quiz questions• FAQ• Module V - Workflow• Quiz questions• FAQ	<ul style="list-style-type: none">13 minutes presentation14 minutes presentation12 minutes presentation15 minutes presentation14 minutes presentation
Practical trainings	<ul style="list-style-type: none">Introduction to Practical trainingDonning & DoffingSample taking & rapid test	<ul style="list-style-type: none">10-20 minutes1 hour1 hour and 30 minutes

	Standard Operation Procedures	1 hour
	Root Cause Analysis	1 hour
	Design test site	1 hour
	Setup test site	45 minutes
	Simulate test site	1 hour and 30 minutes
Appendix	Materials, supplies, and kits for practical trainings	<i>NA</i>

Chapter 4

Practical information

Presentations can be provided for participants in person and/or online. For **workshops**, it is recommended for optimal learning experience and management that the number of participants do not exceed five (maybe six) participants per instructor. This number is small enough for all participants to be fully engaged, yet large enough for a variety of experiences and viewpoints to be represented.

4.1 Facilities and equipment

For in-person trainings, the environment should be large enough for training, discussion and the simulation. The trainings or workshops can be held in a well-lit, ventilated, distraction-free classroom with sufficient tables and chairs, and conveniently located outlets for a computer and projection monitor. To facilitate discussion and interaction among participants, tables should be arranged in a semi-circle, or classroom-style, giving all participants an unobstructed view of the projection monitor. Avoid overcrowding. It is important to consider social distancing requirements when preparing the training venue and limit the training to smaller groups. Bottled water and glasses should be made available on each table. Facilities must be available for participants to clean their hands (with soap and water or an alcohol-based hand rub). Refer to local guidelines for measures that must be in place for workshops of this nature.

Make arrangements well in advance of the workshop to procure or secure the necessary materials, supplies and kits. Do not forget to arrange for transport of these items to the workshop site.

4.2 Target audience

The training can be provided to lab technicians, lab managers, first responders, quality managers, or anyone involved in SARS-CoV-2 screening. The setup and content of the training is adequate for all backgrounds and levels of expertise. The training participants should have general knowledge of diagnostic settings and familiar with general aspects of infectious diseases and hygiene.

4.3 Course Language

Training material are provided in English. The documents and forms can be edited for translation into different languages.

Chapter 5

Presentations

5.1 Introduction

The presentations and training material are deployed online on the RIVM **Capp-Agile website**. Participants can access the modules after registration.

A face-to-face meetup can be organised for the participants for in-depth and interactive training, and for discussions with the local training. The trainer can use the presentation sheets and the narrative to compose their own presentation(s). Presentation can be alternated with practical exercises listed in section ‘Practical training.’

The following section lists the content of the presentations, the narratives and in-depth questions and frequently asked questions.

5.2 Module II - Types of SARS-CoV-2 Tests

5.2.1 Content

Length	13 minutes presentation
Learning goals	The participants can: Describe different types of rapid tests for SARS-CoV-2 detection. Argue the (dis/)advantages of the types of tests Illustrate criteria that can be applied to rapids tests Argue why a validation procedure is needed.

Summary	The presentation starts with illustrating the importance of validating new medical devices before they are applied. Does the rapid test fulfill its' role in its' purpose? List types of diagnostics and its characteristics: 1) PCR, 2) Antigen testing, 3) Serology, 4) Biomarkers. Characteristics include price, equipment, sensitivity/specificity, type of sample. As an example, criteria are listed to which both the manufacturer and the tests characteristics need to comply with.
Tools & setup	Ask participants to turn off (sound of) mobile telephones Explain if and when questions can be asked. Relevant literature. PowerPoint slides. Presenter in front of slides. Small quiz for recap.

5.2.2 Narrative

Sheet 02 - Introduction

Training topic

What types of rapid tests are available for SARS-CoV-2 detection?

Why do we need validation?

Presenter: Robert ten Hove

SCK-CEN, European Institute for Public Health and the Environment, Istituto Superiore di Sanità

SAFE

ALESSANDRO VOLTA

04/05/2021

In this module, I will discuss different types of rapid tests for the detection of Corona infections. Countries across the world are facing difficulties containing COVID outbreaks. Testing and contact tracing have a large impact on reducing transmission of the virus. To strengthen the identification of people carrying the virus, there is a large demand for rapid tests, which are also called lateral flow tests. Manufacturers are competing on the market with different types of rapid tests, assuring that their product is of the highest quality. So, if manufacturers are confident in the performance of their tests, do we need to validate them

ourselves? Before we handle this question, let us first examine what types of rapid tests are available and what their advantages and disadvantages are, and what they can be used for.

Sheet 03 – Learning Objectives

The slide has a dark blue header with the title 'Learning objectives' in white. On the left, there's a logo for 'Centres of Excellence' and the European Union flag. On the right, there's a UNICEF logo. The main content area contains the following text and list:

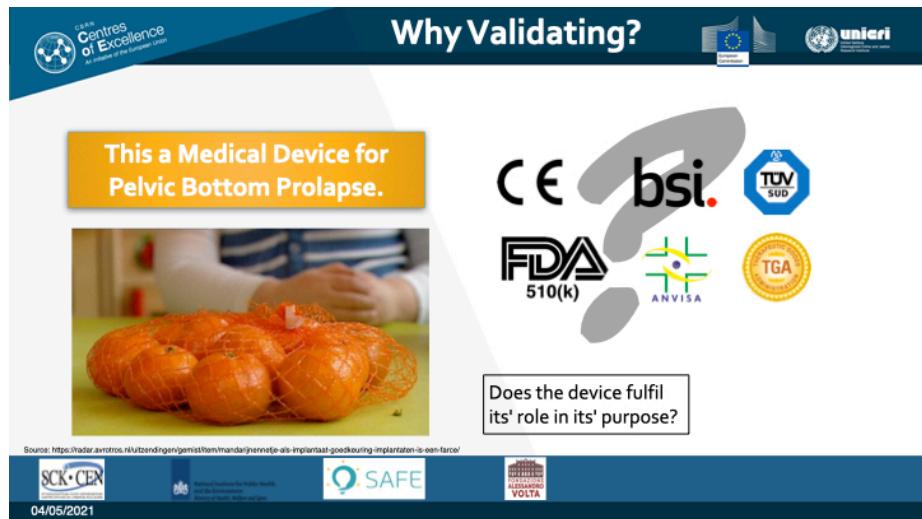
After this presentation you can:

- Describe different types of rapid tests
- Know the (dis)advantages of different tests
- Illustrate compliance criteria for tests
- Explain why a validation procedure is needed

At the bottom, there are logos for SCK•CEN, National Institute for Public Health, and Istituto Alessandro Volta, along with the date '04/05/2021'.

This presentation will take around 15 minutes. We will discuss the basic principles of current Coronavirus tests and their advantages and disadvantages. Different types of tests also have different compliance criteria. The type of compliance criteria depends on how and for what the test is used for. By the end of this module, you will understand why test validation is needed. In the next module, we will learn about the validation procedure itself.

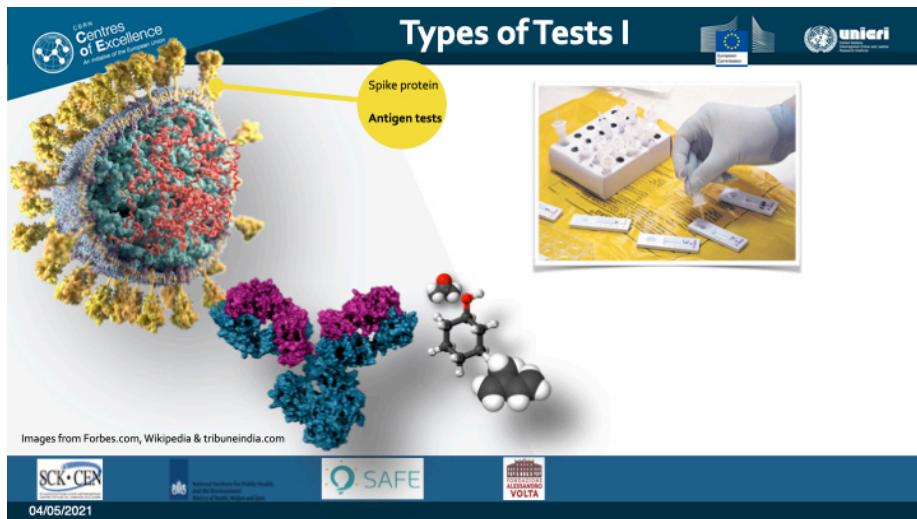
Sheet 04 – Why validating?



Scandals on inferior medical devices are unfortunately common. How is it possible that such devices are still being sold? How does this system work and where do the responsibilities of different stakeholders start and end?

As an example, in 2014, journalists made up a medical device, a Pelvic Floor Net for the treatment of Pelvic Organ Prolapse. The product was a fruit net for oranges. The journalists wrote a brochure and a technical briefing for it. The conclusion was that their product met the certificate requirements for medical devices and was allowed to be sold on the market. This is an extreme example, but it does expose the shortcomings of regulatory affairs. That being said, if regulatory affairs are too strict, it would be very hard to manage outbreaks such as the one we are facing now.

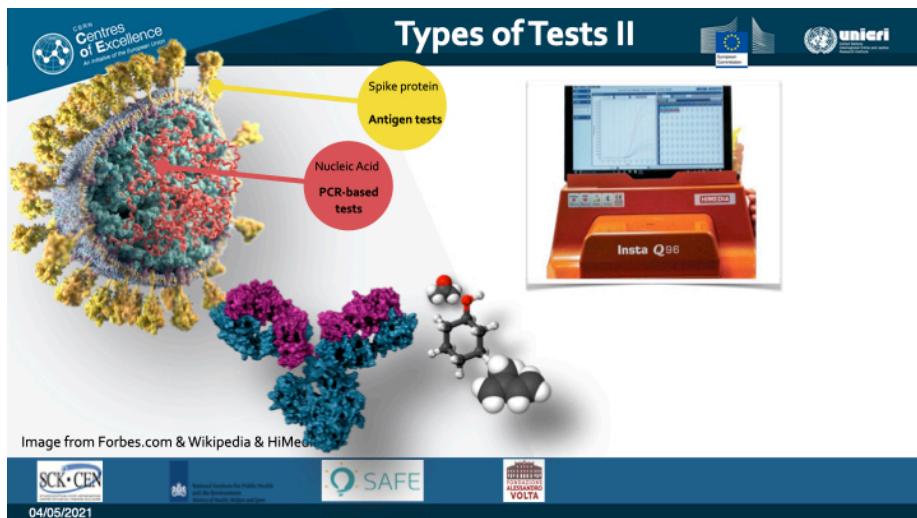
For diagnostic tests being bought to market during the ongoing COVID-19 outbreak, quality control markings do not always guarantee a reliable test as one type of test may work better in one setting and not in another. Therefore, the test needs to be checked, or validated, to see if it works in a slightly different setting and circumstance.



Different types of SARS-CoV-2 tests have different purposes.

In general, there are four types of tests for the detection of SARS-CoV-2. On the left, you see a cross-section of the Coronavirus. The virus particle has these yellow spikes on the outer membrane sticking out. These spikes can be captured by antibodies that are attached to a surface and subsequently coloured with a dye. A well-known example are these rapid lateral flow tests. (*Show rapid test for the camera*).

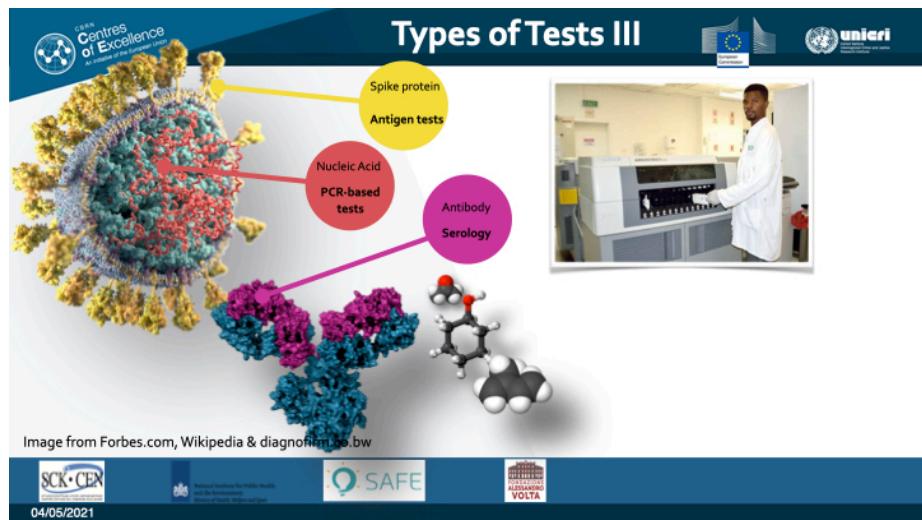
Sheet 06 – Types of Tests II



These spikes are on the outside. To reach the inside of the virus, the particle

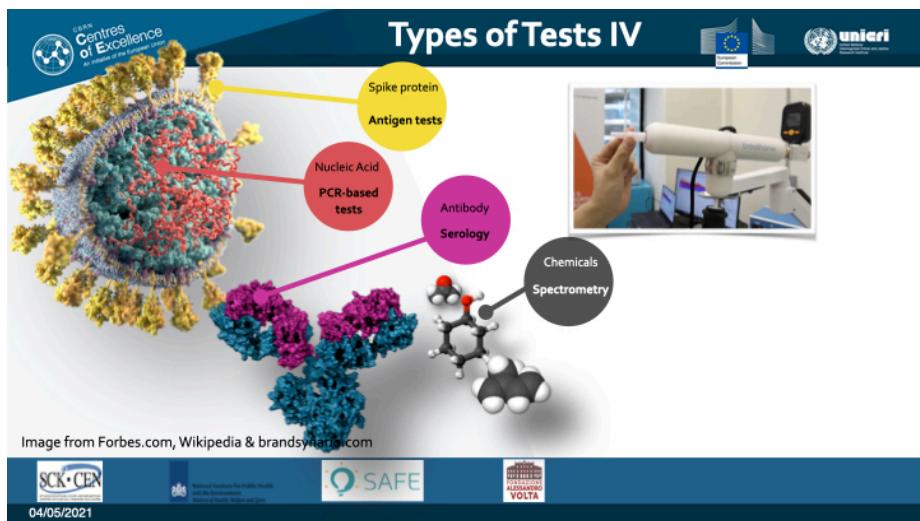
needs to be treated with specific reagents in a test tube. The released genetic material of the virus can then be detected using the Polymerase Chain Reaction analysis, or PCR test.

Sheet 07 – Types of Tests III



In a later stage of virus infection, the human body's immune system is catching up with the infection and is releasing specific antibodies in the bloodstream. These specific antibodies can be detected with the so called serology tests. This is an indirect detection approach for exposure to the virus as it detects human produced antibodies and does not detect the virus itself.

Sheet 08 – Types of Tests IV



Other kinds of molecules or biomarkers are released by the human body during an infection. These molecules can be detected and recognized with sensitive equipment. For example, spectrometry analyzers are used for the detection of specific quantities of small volatile molecules such as acetone, ethanol, and others. Other biomarker tests are biochemical tests include C-reactive protein, measures of anticoagulation or blood clotting, and immune cells such as white blood cell count. Then there also imaging-biomarkers, such as X-ray or CT-scan. These are commonly available tests and may be helpful for the triage of people with possible COVID-19 in imaging the lungs

Antigen and PCR tests can directly detect SARS-CoV-2 viral particles, while serology and biomarkers are indirect detection methods and rely on how the human body reacts to infection. They each have their advantages and disadvantages, depending on what they are exactly used for.



	Purpose	Time	Costs	Specimens
PCR-based	Clinical diagnostics	~1 - 48 hours	~\$10 - 50 + equipment	Naso-opharyngeal, feces & other
Serology	Screening & Monitoring	10 minutes / 24 hours	~\$10 + equipment	Blood (serum)
Antigen test	Point of Care & Screening	20 minutes	\$2 - 5	Naso-opharyngeal
'Other'	Point of Care & Screening	1 minute	\$ 1 + equipment	Breath, body-odor, ?



04/05/2021

Let's compare these different types of tests.

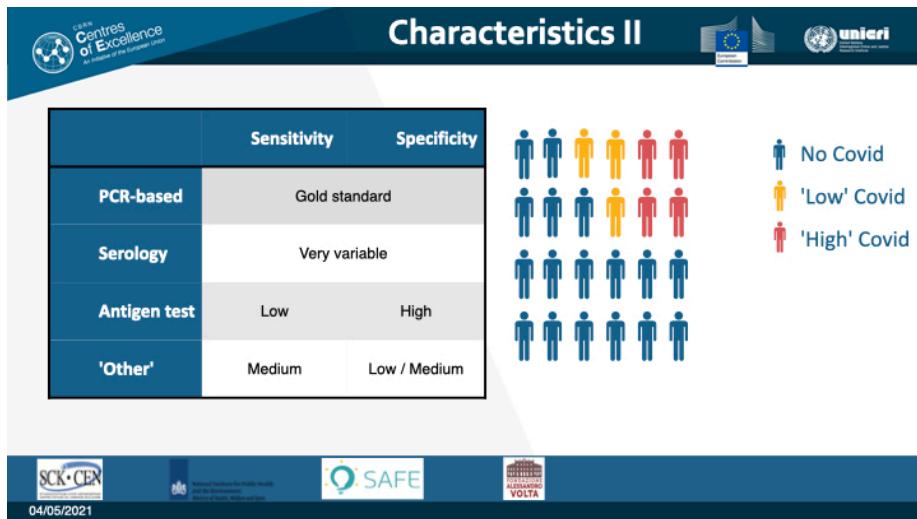
Molecular or PCR-based tests are considered the gold standard. They are versatile and the most reliable test for diagnosing COVID patients. Usually, these tests take a day: samples need to be transported to a laboratory. Then the samples are processed, analyzed, checked, and double-checked. Test reagents and equipment are expensive. Therefore, a PCR test is only cost-effective when they are performed in large quantities. Some PCR tests are less expensive or quicker, such as LAMP and the GeneXpert. Still, these tests require specialized expertise and lab equipment.

Serology is less expensive compared to molecular tests. In general, serology is not used for the first diagnosis of COVID infection. Antibody tests are likely to be available in laboratory form using enzyme-linked immunosorbent assay, or ELISA methods. But also as a point-of-care test, using one or two spots of blood from a thumb prick on a testing strip. It takes around 10 minutes for a positive answer.

Contrary to the antigen test. They are quick, cheap, and require little training. The price, however, is that these rapid antigen tests are less reliable.

Several other rapid tests are being developed, validated, and applied. It ranges from breath analyzers to sniffing insect bees. I have grouped these with '*Others*'.

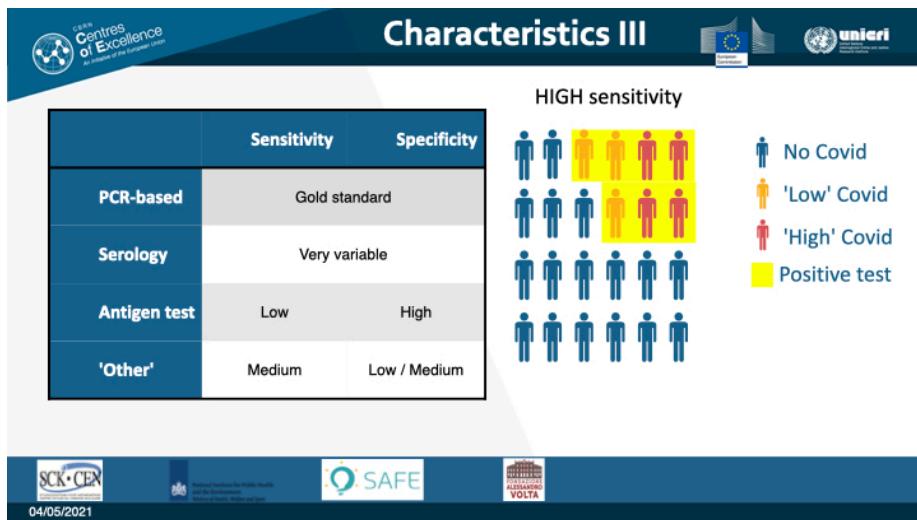
Choosing the right testing method depends on the objective of the test and the available resources. Is for diagnosing a patient? Or for monitoring donated blood units?



The most important indicators for the reliability of a test, are the sensitivity, and specificity of the test. Let's imagine we have a population. Most of them, the blue ones, do not carry the Coronavirus. The yellow ones carry the virus but don't know this yet. The number of viruses they have is very low and they do not feel sick.

The red ones have high viral loads and are really sick with symptoms.

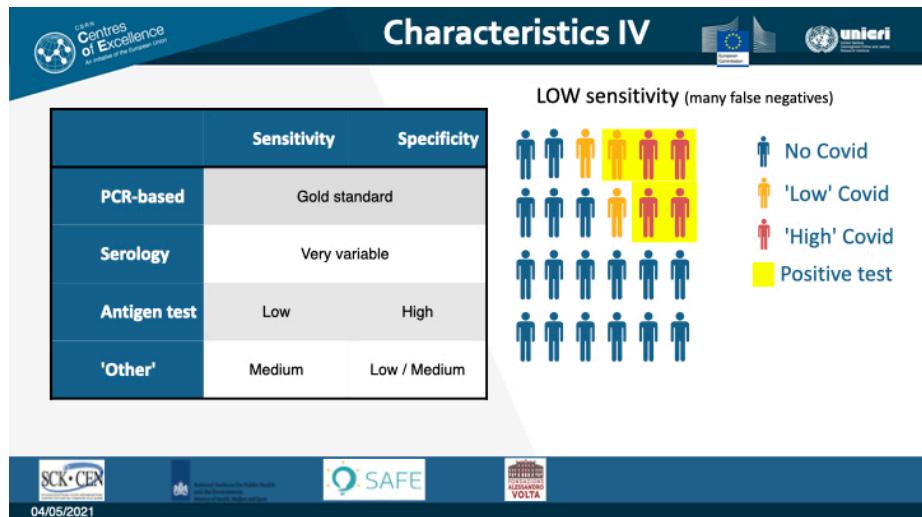
Sheet 11 – Characteristics III



The PCR test performs very well in being 'positive' with persons carrying the virus. Including the ones that are not sick. The test has excellent sensitivity.

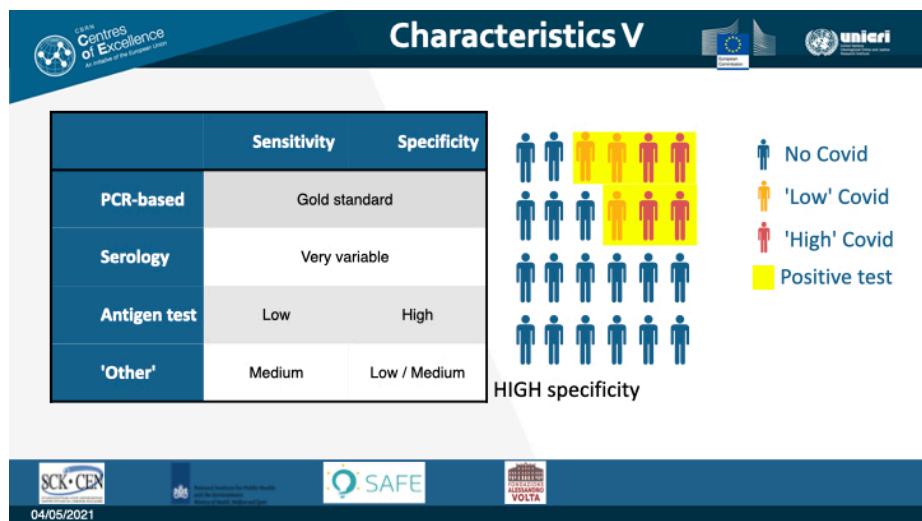
There are hardly any false negative patients.

Sheet 12 – Characteristics IV



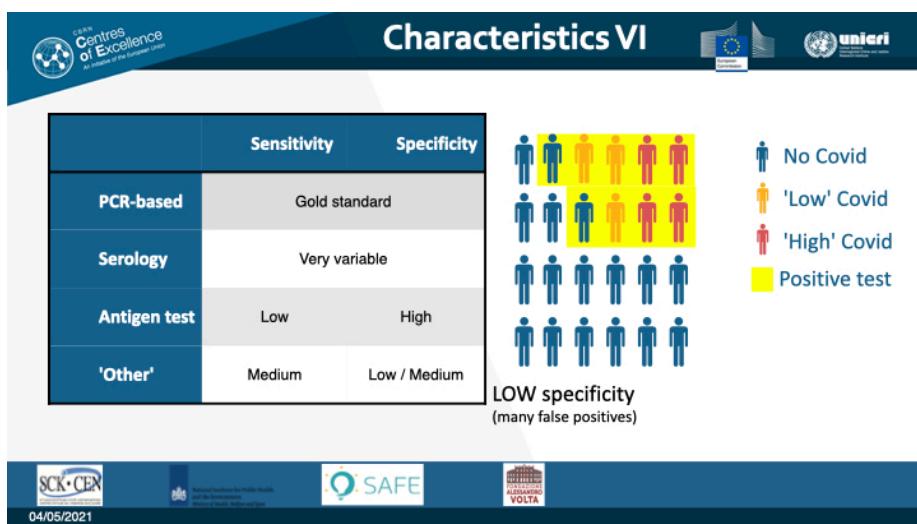
In another type of test, those persons that carry a low number of viruses can be missed when the test has a low sensitivity. This type of test is definitely not recommended in a setting with vulnerable persons, for example in a maternity ward or an elderly home.

Sheet 13 – Characteristics V



With the uninfected people, here, the test result is always '*negative*.' Hardly anyone gets a false-positive result. The test has a great Specificity.

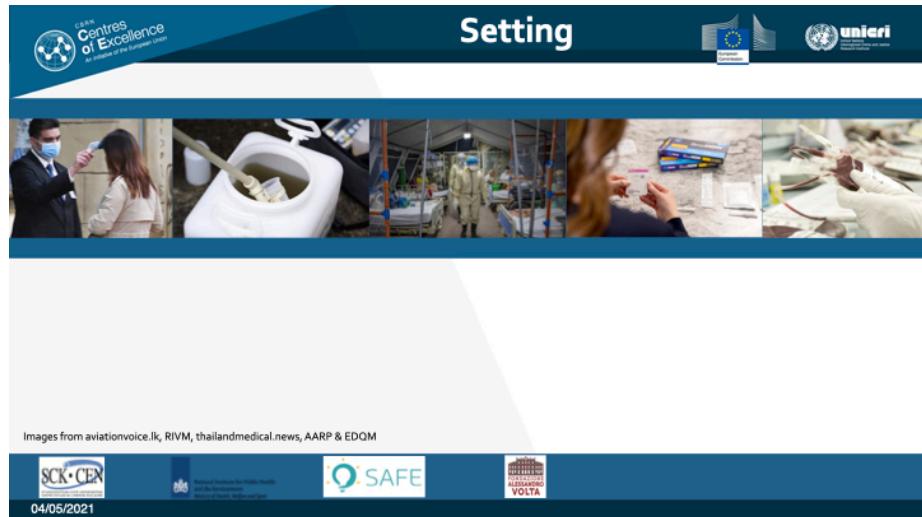
Sheet 14 – Characteristics VI



Low specificity comes when negative persons receive test result which says they positive for the infection with the virus. Now, this does not immediately mean that this test is useless. If this test is quick and cheap, the '*negative persons*' can carry on. The positive ones, need to be double-checked with a more reliable test. This, for example, would work in a setting for screening travellers.

For the sensitivity, it's always better to have it as high as possible. But in some settings, one has to make a trade-off. Less sensitivity for a faster and cheaper test. This is a complicated decision.

Sheet 15 – Setting



As showed, all types of tests have their advantages and disadvantages. The first step before introducing a new test is to ask a few basic questions:

For who is the test? Do we need to quickly screen passengers? Do we need to diagnose a sick person? Do we need to monitor the environment or food products?

What kind of samples are available? Throat or nose swabs? Feces? Wastewater?

Where is the test conducted? At a laboratory? In a mobile health unit? At home?

These questions are all part of formulating the objective when a new test is to be validated.

Criteria

The test needs to:

- be compatible with equipment
- have a minimum sensitivity & specificity
- have a certain availability
- have a maximum price
- be registered & custom clearance
- ...?

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04/05/2021

Once it is clear what the setting is, the list with tests can be further shortened by setting several criteria. Do new reagents work with the equipment already in the lab? What does the manufacturer state about the sensitivity and specificity? And will the manufacturer be able to provide enough test reagents for the near future? Also important is if it is affordable? Are there any legal obligations before the test can be purchased? Are the correct storage options available? This information is all part of the validation plan.

Sheet 17 – Planning of Validation I

Planning of Validation I

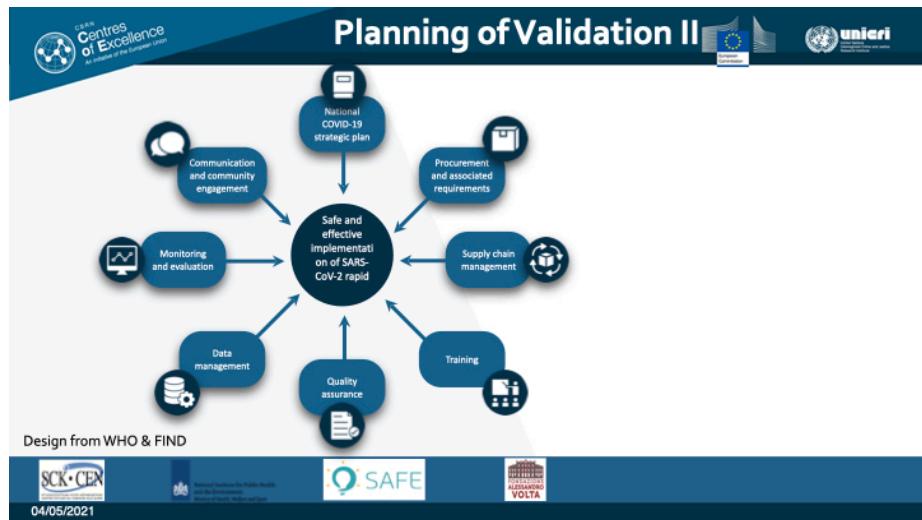
SCK•CEN **National Institute for Public Health and the Environment** **SAFE** **INSTITUTE FOR POLYMER AND VOLTA**

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The reliability of a test does not only depend on the test itself. For validating

the test, processes associated with the collection and production of the outcome needs investigation. It has to be confirmed that all processes provide sufficient assurance that the results are reliable. The validation also justifies why specific processes are needed. Now, it is impossible to put all these processes into one validation.

Sheet 18 – Planning of Validation II



There are many actors involved in the successful implementation of a test. For example, if storage at customs was inadequate, the reagent might have deteriorated and can have a direct impact on the reliability of the tests. The lab may validate as much as they can, the test results remain unreliable. Therefore, it can be very rewarding to sit down with relevant stakeholders and put yourself in their situation. It gives a better understanding of all challenges. By facing the challenges together, you might come up with an alternative for choosing the appropriate test.

Sheet 19 – Summary

Now you can:

- Describe different types of rapid tests
- Know the (dis)advantages of different tests
- Illustrate compliance criteria for tests
- Explain why a validation procedure is needed

Further reading:

- <https://www.who.int/publications/i/item/g789240017740>
- <https://www.finddx.org/sarscov2-eval-antigen/>

There was an overview of the types of tests that are being applied for detecting SARS-CoV-2. It is made clear that one cannot just simply compare one test with another. Especially when they are applied in different settings.

5.2.3 In-depth questions

5.2.3.1 Question 1

Throat swab: or oropharyngeal samples can be used for PCR-based assays to detect the viral RNA, and for antigen rapid tests for the detection of virus-proteins.

Serum or plasma: antibodies in plasma and serum can be detected with serology assays. ‘PCR-assays’ can be used to quantify viral RNA in blood, however, it is less sensitive and more invasive compared to oropharyngeal- and nasal swabs.

Breath sample: detects volatile molecules / chemicals. Usually used for fast screening of ‘negative patients,’ as the test result can be often ‘false positive.’

Nose sample: or nasal sample are used for antigen- and PCR-test assays.

Environment / waste water: these samples are collected to monitor the amount of viral particles from human excretion. Viral RNA is extracted from the waste water and detected with the sensitive PCR-assays.

Blood transfusion: samples are usually analysed with serology for the detection of antibodies for monitoring the prevalence in the human population. Active screening of blood products with PCR is not recommended as 1) blood donor’s are asymptomatic during donation, 2) viral loads in blood with COVID-19 are usually low, in particular with asymptomatic persons and 3) the virus is deactivated during the processing of blood-plasma.

Question 1

Connect types of samples with type of test. (more than one match is possible)

Sample Type	Test Method
Throat swab	Serology
Serum/Plasma	PCR-test
Breath sample	RDT
Nose sample	RDT
Environment / wastewater	Spectrometry
Blood transfusion	Spectrometry

For example....

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Figure 5.1: Question 1

Question 1 - answer

Connect types of samples with type of test. (more than one match is possible)

Sample Type	Test Method
Throat swab	Serology
Serum/Plasma	PCR-test
Breath sample	RDT
Nose sample	RDT
Nose sample	Spectrometry
Environment / wastewater	Spectrometry
Blood transfusion	RDT
Blood transfusion	Spectrometry

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Figure 5.2: Answer 1

5.2.3.2 Question 2

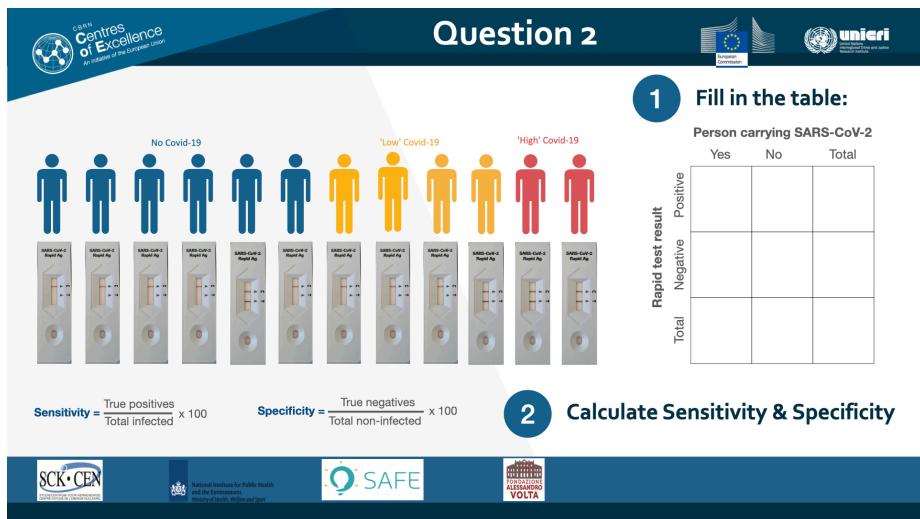


Figure 5.3: Question 2

For the sensitivity of 50%; of all persons carrying the virus, in only half of them the test became positive.

Regarding the specificity of 83%; in all six persons that do not carry the virus, in one person the test became 'false positive'. The study population of 12 is too small to draw reliable conclusions on sensitivity and specificity.

5.3 Module III - Validation of test kits

5.3.1 Content

Length	14 minutes presentation
Learning goals	The participants can: create a validation report

Summary	Steps for validation of a rapid test are explained. <ul style="list-style-type: none"> • Rationale: why is this validation needed for? For example, conventional tests are expensive and shortage of consumables. • Objective: What is the primary (and secondary) question that needs to be answered? For example, what is the sensitivity? • Study population: Rapid tests are validated on well-defined user group. For example persons with COVID symptoms from the age of 16+, willing to participate, etc. • Methods: Ethical review, Statistics, primary & secondary endpoints (use dummy graphics), procedure (questionnaire, sampling, type of tests, storage, transport, testing) • Main study endpoints: explain what will be measured? What is the reference standard?
Tools & setup	Ask participants to turn off (sound of) mobile telephones Explain if and when questions can be asked. Relevant literature. PowerPoint slides. Presenter in front of slides. Small quiz for recap.

5.3.2 Narrative

Sheet 03 - Training topics II

Source: movie Matilda

Training topics II

Does the device fulfil its' role in its' purpose?

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MILIEU- en GEZONDHEIDS VOLTA

In the previous module, the main message about using new tests is that one should not work blindly on promises of the manufacturer or salesperson.

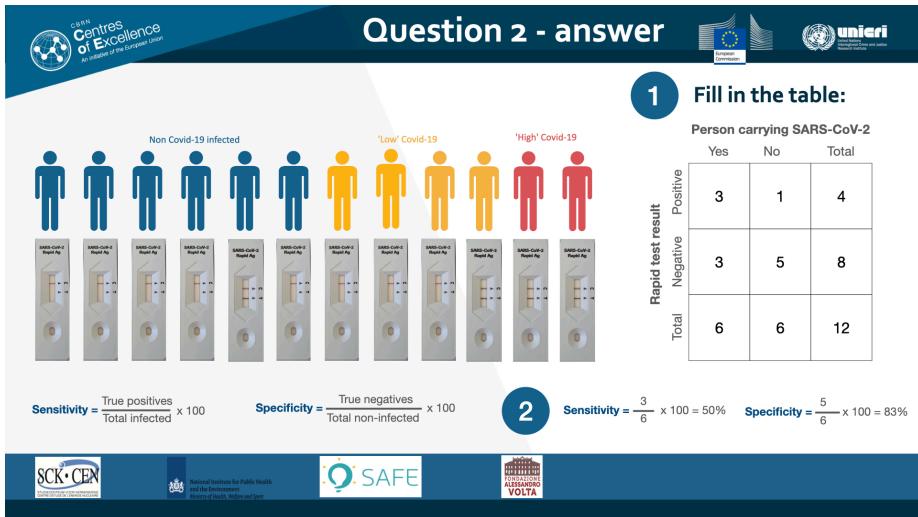
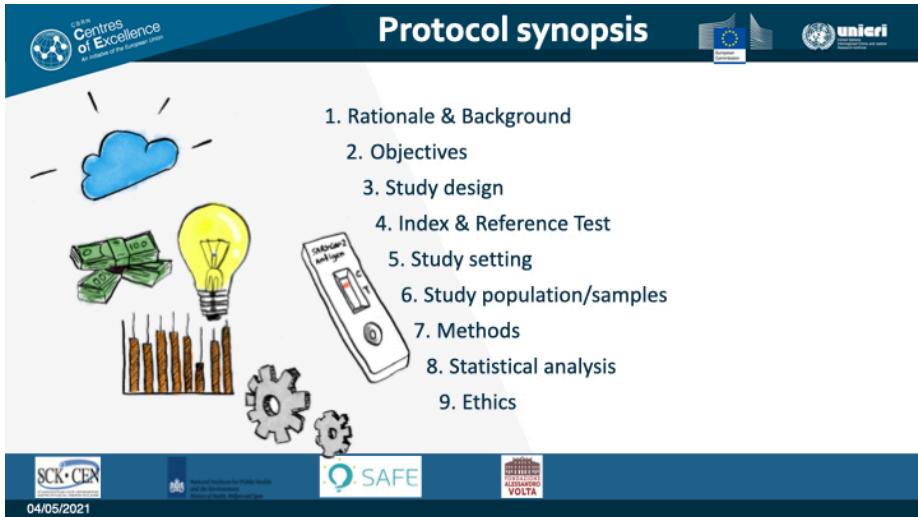


Figure 5.4: Answer 2

With a validation plan, we want to measure if the promises made by the manufacturer meet real-life situations.

Sheet 04 - Protocol synopsis



These are the steps that will be briefly discussed. Of course, a plan does not need to be limited to these chapters. The first chapter is about explaining why the validation is carried out. The Objectives state what exactly is going to be

measured. The Study design is how the study is set up. Chapter 4 explains which tests are going to be used. Chapter six, who are the subjects and what type of samples are taken. In methods, the procedures are explained in detail. For chapter eight i will not explain the methods, but I'll illustrate the input and output of data. Last but not least, the ethical aspects of the study.

Sheet 05 - 1. Rationale & Background

- Increase screening
- Decrease costs
- New test
 - Measure diagnostic accuracy in ...
 - ... pre-/asymptomatic close contacts
 - ... pre-boarding Covid-spreaders
 - Nose and/or throat swabs

During the SARS-CoV-2 outbreak, the laboratory capacity quickly runs to its limit and reagents were getting scarce. The time between getting a patient sample until giving out the test result, also became an important factor in the fight against COVID. Besides time, also costs needed to go down. These are clear rationals to implement new rapid tests: increase the speed of testing and decreasing costs. Once a manufacturer or distributor of a new test is appointed, the test itself should be verified. The manufacturer might promise 99% sensitivity, however, it might have been tested on sick hospital patients shedding high loads of viruses. In your case, you might want to apply the test on asymptomatic persons that had close contact with an infected person. [PHOTO] Or, at the airport for pre-boarding COVID spreaders, you might want to replace the body thermometer with a more sensitive breath analyzer. [PHOTO] Validation can also be set up if a current process needs to be adapted. For example to check if there is a difference in sensitivity if one swab from the nasopharynx has the same sensitivity as to when taking a double nasopharynx swab together with a throat-swab. [PHOTO]

The rationale is to convince others why a change or investment in a new method is needed.

Sheet 06 - 2. Objectives I

2. Objectives I





Primary objective:
"To determine the diagnostic accuracy (sensitivity, specificity) of rapid antigen tests, with RT-PCR as reference standard, in the target population of(?) and who were light-/asymptomatic at the time of test request."



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Suppose we want to check a new rapid test, the main objective is to check the sensitivity and specificity of the test. How many false positives and false negatives are detected with this new test? Here, it needs to be clear who exactly the subjects are, and what kind of samples are being used. It also needs to be clear what the reference method is. The reference can be supplemented with more criteria, such as subjects with specified clinical symptoms.

Sheet 07 - 2. Objectives II

2. Objectives II





Secondary objectives:



- ➡ "Motivation of participants to get tested."
- ➡ "How participants are informed about the test location."
- ➡ "Correlation of Ct-values with rapid test results."

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The experiment could be a good opportunity to gather more information, be-

sides the test itself. For example, a questionnaire could show that many persons get tested is because they think they got infected with SARS-CoV-2 from 5G radiation. In that case, a lot of resources could be saved by starting an information campaign.

Correlation with Ct-values means if there is a pattern the positive and negative outcome of the rapid tests, and the strength of the PCR signals, which is expressed as Cycle-threshold values, or Ct values.

Hence, the objective of the plan is the research question, or the hypothesis: what exactly do I want to measure or reproduce?

Sheet 08 - 3. Study design

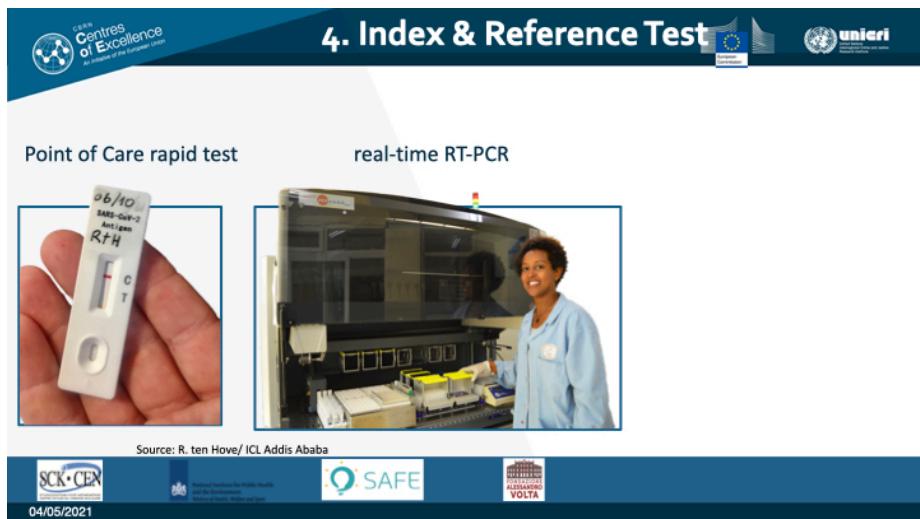


The chapter on study design is a more technical explanation of how the experiment is set up. In general, a test validation can be conducted in a completely controlled environment in the laboratory. There is total control over the parameters: samples were stored in the freezer and concentrations of virus in the positive samples are well-defined. The negative samples could be spiked with several respiratory viruses, other than SARS-CoV-2, to check if the tests show cross-reaction with other viruses. The test reagents are stored at the same temperature and the equipment is handled by one researcher. The lab-based performance or technical validation is usually already performed by the manufacturer.

In a clinical evaluation, there are many more uncertainties, a good representation of real-life. Samples are taken from multiple sites, by different persons, over a period of time, from unknown test subjects. The study is blinded, which means that the technicians who are testing the samples do not know if the

samples are positive or negative. The results are compared by an independent person.

Sheet 09 - 4. Index & Reference Test



Index and reference test. In short, describe the test or maybe several tests that need to be validated, and to what the method is being compared to. It must be taken into account that, although PCR is considered the gold standard, it is also not 100% sensitive and specific.

Sheet 10 - 5. Study setting

5. Study setting

Source: Jeff J Mitchell/Getty Images Source: R ten Hove / Corona Testzentrum Kranenburg

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In this chapter, it is described where the samples are coming from. Are the swab samples and data coming from one site or multiple sites? It might be important when the demography of the subjects is described. A testing centre at the university campus will provide samples from more young persons, and thus likely to have samples with lower viral loads.

So, location can also be important for the interpretation of the results.

Sheet 11 - 6. Study population

6. Study population

Inclusion criteria:

- Aged ≥ 16 years
- Consent for participation

Exclusion criteria:

- Not meeting criteria listed above
- Hospital admitted

Sample size

- Minimum of 100 Covid RT-PCR positive; minimum of 100 Covid RT-PCR negatives
- Time period...?

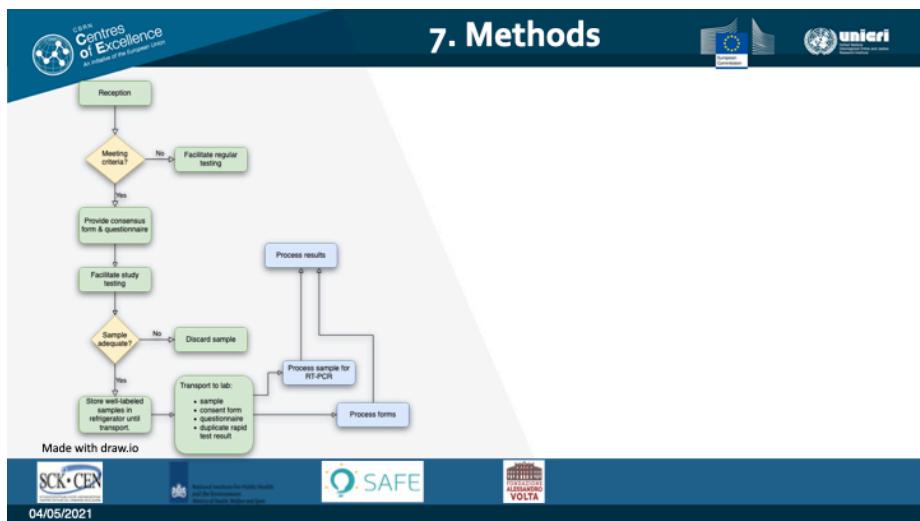
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As mentioned in the previous sheet, age can be an important factor and needs to be recorded to assure that the sample population is a representative cross-section

of the general population. The same applies to gender. This chapter also needs to describe the inclusion criteria. For example, children until 16 are excluded. Also, persons that are mentally or physically unable to provide consent, should be excluded. The test is validated for asymptomatic persons, therefore, subjects need to be excluded from the study when they present COVID symptoms. Basically, what is needed is a homogenous study population that represents the study objective.

Sheet 12 - 7. Methods



Here, a diagram shows all the steps during the study. It starts when the subject presents itself at the reception, all the till the final analysis of the test results. The chapter also describes the procedures, among other how the swabs are taken and the PCR analysis. These procedures can also be described in validated standard operation procedures, which I will discuss in my next module. Different forms, such as the questionnaire and the consent form can be added as an appendix to the validation plan.

Sheet 13 - 8. Statistical analysis I

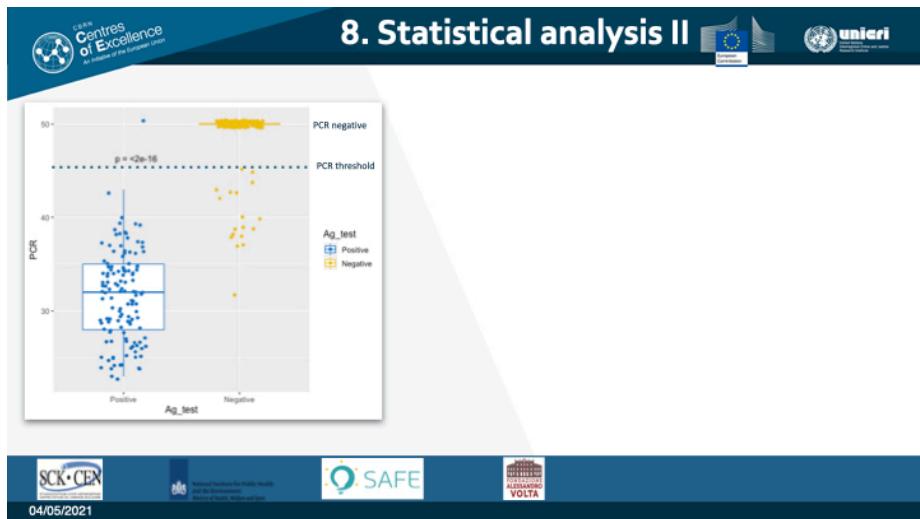
8. Statistical analysis I

Variable	Description	Data type	Range	Unit
Gender	male / female / other	factor	0;1;2	0 = male 1 = female 2 = other
Age	for uniformity / prevent bias	numeric	16 - ?	years
Ag_test	antigen test result pos/neg	binary	TRUE/FALSE	
PCR	real-time RT-PCR test result	numeric	12 - 45	Ct-value; 50 = negative
reason	subject reason for testing	factor	1; 2; 3; 99	1 = close contact 2 = pre-travel 3 = other 99 = missing value
informed_by	subject informed by...	character	1 - 255	number of characters (99 = missing value)

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In this module I will not discuss the statistical methods: that would take too long... and boring for some. The important point here is that, it would be very unfortunate if, at the end of the study, it appears that conclusions cannot be made. Simply because we forgot to ask the age of the subjects. How would we know for sure that we have an honest representation of the general population? All work might have been done for nothing.

Another tip is to structure all data. The most annoying thing for me as a data scientist is to clean up data and use my own interpretation on the collected data. For example, if the reason for testing says “Family.” What does that mean? Traveling to family? A family member was COVID positive? Basically, if garbage data goes in, garbage results come out.



Here is an example of the statistical analysis. The numbers for this graph are made up by the way. It's for illustration only. At the bottom of the graph are the results of the rapid antigen test. Blue dots on the left are antigen positive, and the yellow dots are antigen negative. On the left side, it says PCR. Often the PCR results are expressed in Ct-values. The lower the Ct-value, the stronger the signal, meaning that there were many viruses in the samples. So, if the Ct-value has a higher number, it means there were fewer viruses in the sample. It goes up until it reaches a threshold. In this case, the Threshold is value number 45. Above 45, there were no or too little virus particles to be detected. For the data analysis, however, I gave the PCR negative results the artificial Ct-value 50. All test results with Ct value above 45... or if no virus was detected, the sample gets Ct value 50.

In the right column with yellow Antigen negative test results, you can see that many of them still provided a positive PCR result. Most of them were weak-positive, and have a high Ct-value. This is to be expected. In the left column with blue Antigen positive test results, almost all of them were also detected with PCR. There is, however, one exception. One single blue dot above the threshold. The antigen was positive but the PCR was negative. Maybe the PCR test went wrong. There was maybe an air bubble in the reaction tube. Maybe the technician made an error and swapped a sample? Still, I would not worry too much about it with these results.

In any case, making some sketches of possible graphs and tables, before the actual experiment starts, can help to improve the objective and setup of the experiment.



For clinical experiments, consent of the participant is mandatory, at least in most places. Consent is needed to use the samples and information from the participants. But ask yourself: what information do I need to reach my objectives? Do I really need the ethnicity of the participant? Do I need to know their religion? What is the worst case-scenario if all collected information would be stolen?

Another example, suppose the rapid test is negative. But later the PCR shows to be positive. Do I need to inform the patient? A few things to consider: if persons are to be informed, then private contact details will have to be collected. Furthermore, if the PCR analysis is performed two weeks later, is it then still worth tracing back the patient?

Summary

The cover page features the FINDDX logo, the European Union flag, and the UNICEF logo.

Comparative evaluation of lateral flow assay tests that directly detect antigens of SARS-CoV-2

1 Protocol synopsis

Title	Comparative evaluation of lateral flow assays that directly detect antigens of SARS-CoV-2 and can be interpreted visually or through the assistance of a reader
Short title	COVID-19 Antigen RDT Evaluation
Use case of test	Rapid, point-of-care (POC) detection of active infection in adults with suspected COVID-19
Rationale and background	The aim of this study is to independently evaluate the performance of novel, rapid, point-of-care (POC) lateral flow assays for the direct detection of SARS-CoV-2 antigen (Ag) in comparison to the current standard of reference testing, RT-PCR. The protocol aims to evaluate the performance of the lateral flow assays (LFA) RDTs as a lab-based approach using confirmed respiratory specimens; 2) a prospective clinical approach using LFA RDTs to detect active SARS-CoV-2 infection in adults with suspected, confirmed or suspected COVID-19, as defined by national or WHO case definitions.

If you have any comments or questions, please contact us at finddx@find.org.

<https://www.finddx.org/wp-content/uploads/2020/04/20200421-COVID-Ag-RDT-Evaluation-Synopsis.pdf>

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We went through every chapter of the validation plan. The most important points are:

Describe the objective of the evaluation.

Make sure that the setup of the experiment is well thought through, and does not need to be adapted halfway through, because we ran out of one reagent. Therefore, it is important to involve all parties that play a role in the validation. From finance to chauffeur.

Also, statisticians, they can fix mistakes, but only to a certain level. Better to involve them in the set-up.

And last but not least, the ethics involved with the validation.

Question part 1

Positive results from either Ag (antigen) or Ab (antibody) tests, together with the presence of respiratory symptoms indicate that the person is likely to be actively infected with SARS-CoV-2.

TRUE FALSE

Ag (antigen) RDTs can enable fast (15 minutes), decentralized access to testing, but generally have decreased performance compared with lab-based PCR tests.

TRUE FALSE

Ag (antigen) RDTs should be used for seroprevalence surveys to monitor the spread of SARS-CoV-2 in the population.

TRUE FALSE

A patients with a Negative Ab (antibody) RDT can be interpreted as: non-active infection.

TRUE FALSE

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Figure 5.5: Question 1

5.3.3 In-depth questions

5.3.3.1 Question 1

Questions part 1 - Answers

Positive results from either Ag (antigen) or Ab (antibody) tests, together with the presence of respiratory symptoms indicate that the person is likely to be actively infected with SARS-CoV-2.

TRUE FALSE

Ag (antigen) RDTs can enable fast (15 minutes), decentralized access to testing, but generally have decreased performance compared with lab-based PCR tests.

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Although antibodies do not necessarily indicate an ‘active infection,’ in combination with respiratory symptoms does make it very likely.
The lower sensitivity of rapid antigen tests is the trade-off for ease-of-use, low price and fast result.
Not antigen, but antibody assays are used for sero-prevalence surveys.

The sero-conversion (development of antibodies in blood serum) appear several days **after** infection or vaccination. Hence, with the negative Ab RDT, the person can still have a recent infection.

5.3.3.2 Question 2

The interface includes logos for ECDC, UNICEF, and the European Union. A title "Question part 2" is at the top center. Below it is a table with two columns: "Suggested use" and "Case management in active outbreak setting" (row 1) or "Public health measures" (row 2). The "Suggested use" column has three rows: "Triage suspect cases", "Support diagnosis in symptomatic cases presenting late (≥10 days post-symptom onset)", and "Monitor active infection". The "Case management in active outbreak setting" row has three rows: "Screen contacts for infection", "Screen contacts for previous exposure (≥10 days post-symptom onset)", and "Seroprevalence surveys to define levels of population exposure, including vaccine trial support". To the right of the table is a blue box containing the text "Select appropriate test." Two arrows point down from the "Ag RDT" and "Ab RDT" headers to the corresponding columns in the table. At the bottom, there are logos for SCK-CEN, National Institute for Public Health and the Environment, and INSTITUTO SUPERIOR DE VOLTA. The date "04/05/2021" is also present.

Suggested use	Ag RDT	Ab RDT	
Case management in active outbreak setting	Triage suspect cases Positive: no confirmatory testing required Negative: confirmatory testing with PCR recommended, if available.	<input type="radio"/>	<input type="radio"/>
	Support diagnosis in symptomatic cases presenting late (≥10 days post-symptom onset) In addition to PCR/Ag, not a replacement	<input type="radio"/>	<input type="radio"/>
	Monitor active infection	<input type="radio"/>	<input type="radio"/>
Public health measures	Screen contacts for infection	<input type="radio"/>	<input type="radio"/>
	Screen contacts for previous exposure (≥10 days post-symptom onset)	<input type="radio"/>	<input type="radio"/>
	Seroprevalence surveys to define levels of population exposure, including vaccine trial support	<input type="radio"/>	<input type="radio"/>

Figure 5.6: Question 2

This question reflects a situation where PCR-testing is not or limited available.

5.4 Module IV - Quality Management

5.4.1 Content

Length	12 minutes presentation
Learning goals	<p>The participants can:</p> <ul style="list-style-type: none"> • Argue the need of standard procedures. • Judge if a batch can be released. • Evaluate potential risks during the testing procedures.

Summary	Topics of the module include: <ul style="list-style-type: none"> • What is Quality Management and why do we need it? • Why do we need Standard Operation Procedures? (for example for using the rapid test) • Batch release. • Risk assessment.
Tools & setup	Ask participants to turn off (sound of) mobile telephones Explain if and when questions can be asked. Relevant literature. PowerPoint slides. Presenter in front of slides. Small quiz for recap.

5.4.2 Narrative

Sheet 03 - Learning objectives

After this presentation you can:

- Describe what quality management is and why it is important
- Understand the types of inaccurate results and their consequences
- List common errors at rapid testing sites and how to avoid them

First of all, the terms SARS-CoV-2 and COVID may be used interchangeably. SARS-CoV-2 is the correct term for the virus, but I will sometimes abbreviate it simply as COVID.

Second, the focus is mainly on Quality Management for settings where rapid antigen tests are used. Still, the information can also be applied for other settings.

Now, about Quality management. This term might be considered as some upper level vague concept. In reality, quality management affects every single step and procedure inside and outside the laboratory. From receiving the patient or client, to submitting the test result. Any weak link or error in the course of actions could set in a chain reaction with disastrous effects in the end. It can

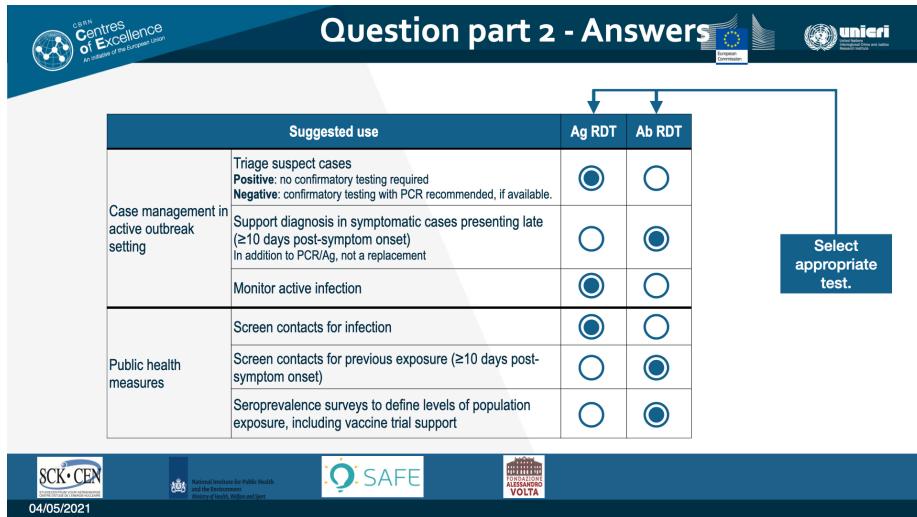


Figure 5.7: Answer 2

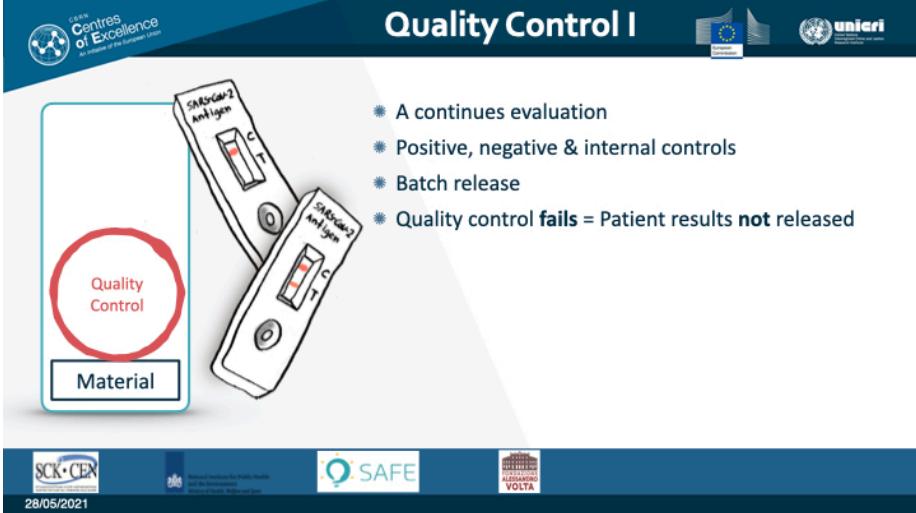
be something small as not monitoring the temperature of the refrigerator, or getting sloppy with personal protection. I will discuss these types of risks, their consequences and how to avoid them.

Sheet 04 - QC/QA/QM



Quality control, quality assurance and quality management, these terms might be confusing. In general, they can be described as follows.

Sheet 05 - Quality Control I



Quality Control I

- A continuous evaluation
- Positive, negative & internal controls
- Batch release
- Quality control fails = Patient results **not** released

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Quality control involves the continuous evaluation or monitoring of the performance of the COVID Antigen Rapid diagnostic tests. This is to ensure that all steps are correctly followed, steps that are required for accurate diagnosis.

Quality control consists of samples from which it is known they are POSITIVE or NEGATIVE.

These reference samples are used regularly in accordance with the manufacturer's instructions and national policy. It is advisable to perform QC testing at least once a week or more frequently if the testing site has conducted a high number of tests.

Another moment to perform a Quality control test is when a new batch or lot number of Antigen rapid tests is going to be deployed. At least five samples (three positive and two negative) should be tested for each new lot. One could add rapid tests both from the ones currently in use, and from the new lot number

Quality control fluids may be supplied with the COVID Antigen test kit. If materials are not supplied with the kit, they can be purchased kit supplier or a third-party provider. Another method is to pool positive and negative patient material. If the quality control results do not match the expected results, Quality control has failed. Investigation and corrective actions are required.

Patient test results cannot be released if quality control fails.

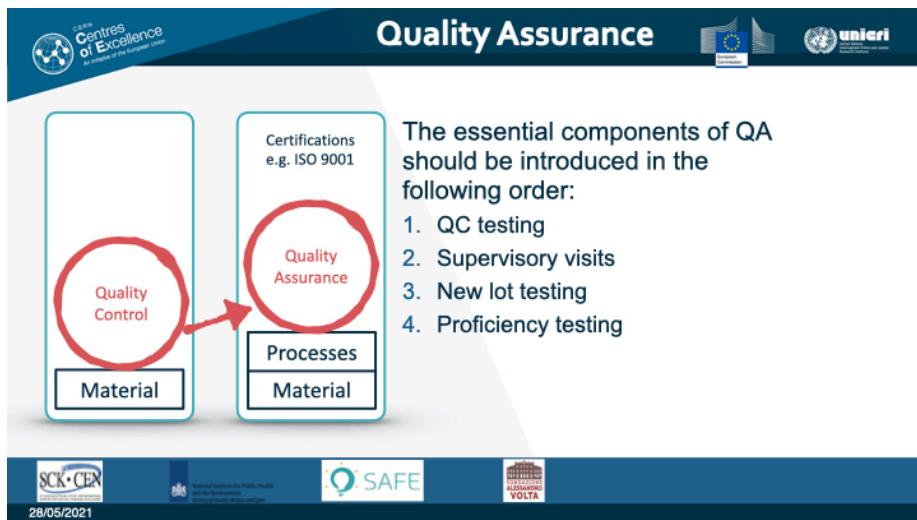
Sheet 06 - Quality Control II



Quality control for the release of new batches does not only apply for the rapid tests. It applies for all consumables, including the swabs, the masks, the disinfection fluid. New material and batches need to be checked and evaluated if they meet the specified requirements.

This is a picture made at a testing site. The wagon containing material that do not pass the quality control. For example these swabs.. [Show swabs that did not pass QC]

Sheet 07 - Quality Assurance



The next level concerns quality assurance. Even if all material passed their

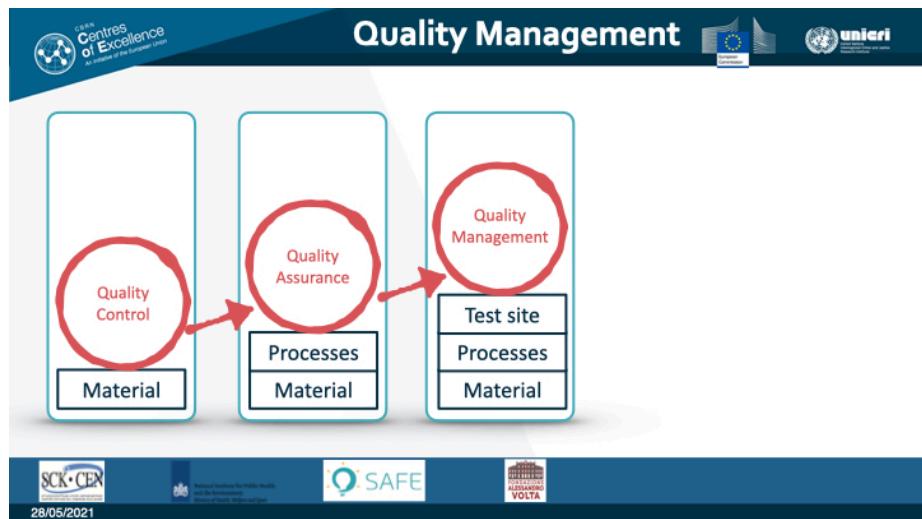
quality control test, if they are used in a wrong matter, the patient can still be sent out with the false result.

Given the need to quickly roll out COVID Antigen Rapid testing, a full Quality Assurance system may not be in place when testing is started. Therefore, to maximize quality, it is essential that a good-quality Rapid Diagnostic Test product is purchased, and then transported and stored according to the manufacturer's instructions. The users must be trained and supervised, but with a mechanism in place for them to report concerns and complaints.

The essential components of Quality assurance should be introduced in the following order:

1. QC testing
2. Supervisory visits or audits
3. New lot testing, or new lot verification of incoming kits
4. Proficiency testing

Sheet 08 - Quality Management



Quality assurance requires a lot of organization. The personnel on the ground do not have time for this. Also they might be biased. Quality management usually in the hands of a dedicated Quality Manager. The manager does not necessarily need to have a technical background, and knows all the in's and out's of the laboratory. The role of the quality manager is to assure that procedures are kept in place. The manager organised the internal audits. The manager receives any incident reports and makes sure they are being followed up.

In short, quality control is about materials, quality assurance how materials are being used, and quality management how everything is organized.

Sheet 09 - EQA

EQA

External Quality Assessment

- Supervisory visits & audits
- Proficiency testing
- Re-testing (!)

photo at ICL, Addis Ababa

UK NEQAS COVID EQA SERVICES PROGRAMMES International Quality Expertise

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There are a few more elements regarding Quality Assurance, which are grouped as External Quality Assurance.

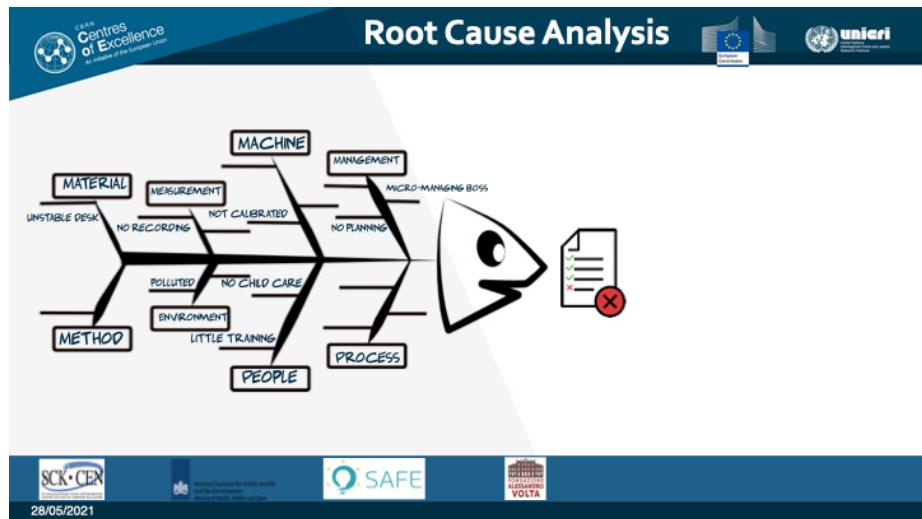
Before the testing site is opening its doors, a supervisory visit can assess the readiness of the place. On the website of this module you can download an example of a testing site readiness checklist.

After the testing has started, the site can be assessed periodically, for example four times per year. Or more often when there are frequently problems recorded. You can also download the testing site supervisory checklist.

A proficiency testing program can be used to identify and resolve problems in diagnostic testing. Well characterized samples can be used for proficiency testing. The sample could for example contain different loads of the virus. Samples can be purchased from external agencies, for example NEQAS. Samples can also be exchanged between testing sites.

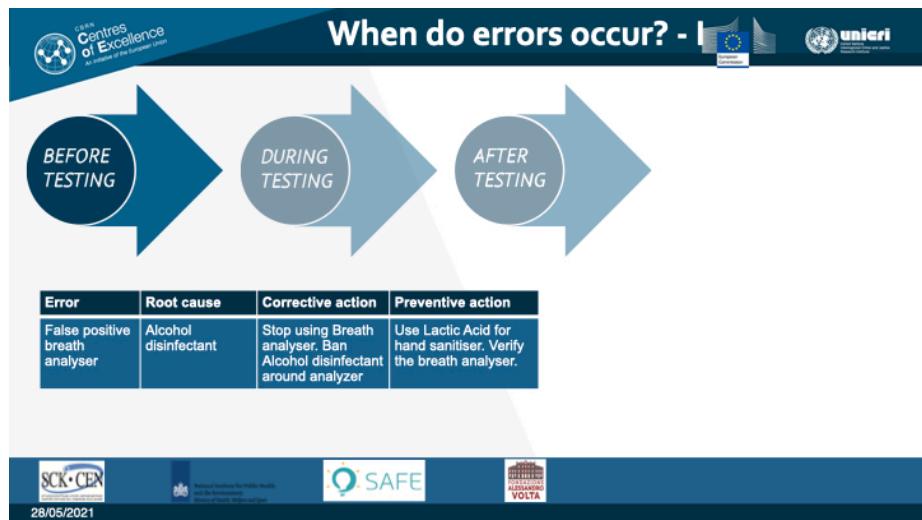
For Quality Assurance, patient samples could also be retested at a different site. However, this is not advisable due to the practicality of collecting multiple samples and the safety associated with their transport.

Sheet 10 - Root cause analysis



Failed proficiency test, or any other major mistake, requires investigation. The reason for the error need to be uncovered. This is called root cause analysis. An example of a tool is this fish bone method, where different areas are systematically examined. When the root cause has been identified, corrective action must take place. Furthermore, preventive actions should prevent the reoccurrence of the mistake. These errors and actions must be documented by the testing site.

Sheet 11 -When do errors occur? - I

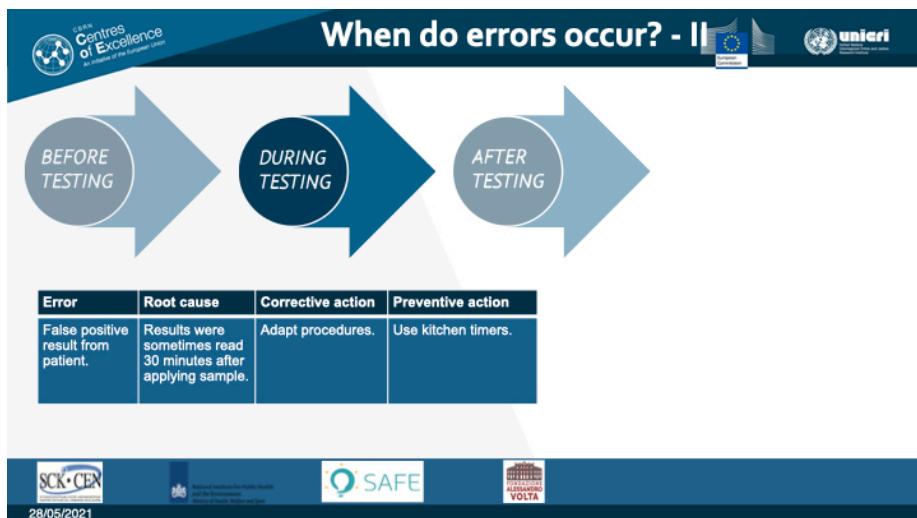


Before entering the testing site, clients need to sanitize their hand with alcohol dispenser. They are first directed to the breath analyzer. If the analyzer is neg-

ative, the client is safe and can go out. If the analyzer result is 'undetermined', the client needs to do a rapid PCR or LAMP test. However, it quickly becomes clear there is something wrong with the analyses. At the end of the day, almost all clients test results become undetermined.

The root cause is the alcohol hand sanitizer. The breath analyzer is too sensitive. The corrective action is to stop using the breath analyzer and direct all clients to the LAMP test. The preventive action is to introduce lactic acid and ban all alcohol disinfectants around the breath analyzer. With the new preventive action, samples are run in parallel both with the breath analyser and the LAMP test.

Sheet 12 -When do errors occur? - II



In another setting where rapid antigen tests are used. It's very convenient as test results are provided on the spot. The number of patients grow quickly, and then the weekly reports show that the percentage of positive patients is increasing significantly, from 12 % to 45%. An increasing number of patients complained that they had to go into quarantine, but never tested positive in the following days. The root cause analysis showed that when there are a lot of customers, the technicians sometimes failed to check the results after 15 minutes. Sometimes the results were checked after 30 minutes. Antigen can become false positive when they are left too long. A solution was to buy the manual kitchen timers and put is with each rapid test. Also, when it becomes busy, the floor manager is helping in the process.

Sheet 13 -When do errors occur? - III

Learning objectives

After this presentation you can:

- Describe what quality management is and why it is important
- Understand the types of inaccurate results and their consequences
- List common errors at rapid testing sites and how to avoid them

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After the testing is done, the test results are shared with the customers. The next day, however, it became clear that the results of two patients with the same name were swapped. One was false positive and went into quarantine. Also, also the close contacts also went for COVID testing. The other person who was tested false negative, became ill the next day. After giving sincere apologies, the close contacts of the COVID patient had to be traced. Clearly, using the patient name as a single identifier is not enough.

Sheet 14 -Summary

Summary

Quality Control
Material

Quality Assurance
Processes
Material

Quality Management
Test site
Processes
Material

- * What are Quality Control, -Assurance and -Management.
- * Tools for Quality Assurance.
- * Root cause analysis

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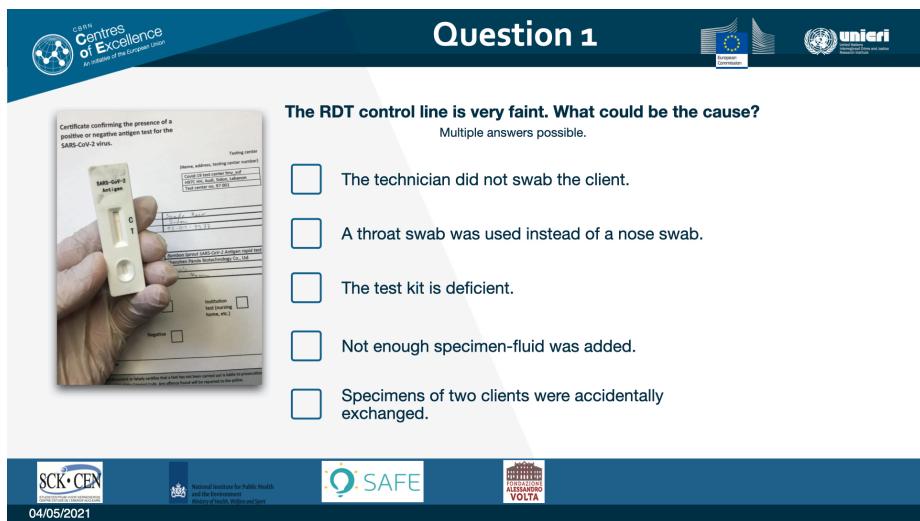
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In this presentation it was explained what quality control, assurance and man-

agement are. Several tools and examples were shown on how to implement quality assurance at a testing site. One of the tools is root cause analysis, for the continues improvement of the testing facility.

5.4.3 In-depth questions

5.4.3.1 Question 1



Question 1

The RDT control line is very faint. What could be the cause?
Multiple answers possible.

- The technician did not swab the client.
- A throat swab was used instead of a nose swab.
- The test kit is deficient.
- Not enough specimen-fluid was added.
- Specimens of two clients were accidentally exchanged.

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Figure 5.8: question 1

‘The technician did not swab the client’ is **false**. The control line detects **any** antibody taken with the swab from the patient. If the client would not be swabbed, there would not be any antibody and, therefore, no control line at all.

A throat swab was used instead of a nose swab; in both cases, human material is taken and the control line should therefore be clearly visible.

The test kit is deficient: This can be the reason. For example a production failure or not well stored.

Not enough specimen-fluid: This can also be the reason.

Specimens of two client exchanged: **false**. Both clients should provide a positive control band.

Question 1 - answer

The RDT control line is very faint. What could be the cause?
Multiple answers possible.

- The technician did not swab the client.
- A throat swab was used instead of a nose swab.
- The test kit is deficient.
- Not enough specimen-fluid was added.
- Specimens of two clients were accidentally exchanged.

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Figure 5.9: answer 1

5.4.3.2 Question 2

Question 2

Often, the control lines appear to be very faint.

What could be the root cause for this?

Question 2 - Answer

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This question goes deeper in the issue with faint control line. This is an open question where several possible root causes are listed as examples.

5.5 Module V - Workflow

5.5.1 Content

Length	15 minutes
--------	------------

Learning goals	The participants can: Design a proper and safe workflow in a routine testing facility.
Summary	Proper registration procedures are explained to avoid mis-diagnosis. Biosafety work procedures for the protection of staff, clients and environment, which also include safe discard of waste. Examples of crowd control methods are shown to improve the testing-flow and a safe environment for the clients and staff.
Tools & setup	Ask participants to turn off (sound of) mobile telephones Explain if and when questions can be asked. Relevant literature. PowerPoint slides. Presenter in front of slides. Small quiz for recap.

5.5.2 Narrative

Sheet 03 - Learning Objectives

After this presentation you can:

- Design a proper and safe workflow in a rapid testing facility

After this presentation, you will be familiar with the design of a proper and safe workflow in a rapid testing center. Safe for both the clients and the staff. Not all details can be covered in a quarter of an hour. Still, hoping that by showing some examples, it will inspire you and you will come up with new ideas and solutions to improve the testing facility.

Sheet 04 - Testing center area

When a large new testing center is set-up, it might be naturally to fit it with

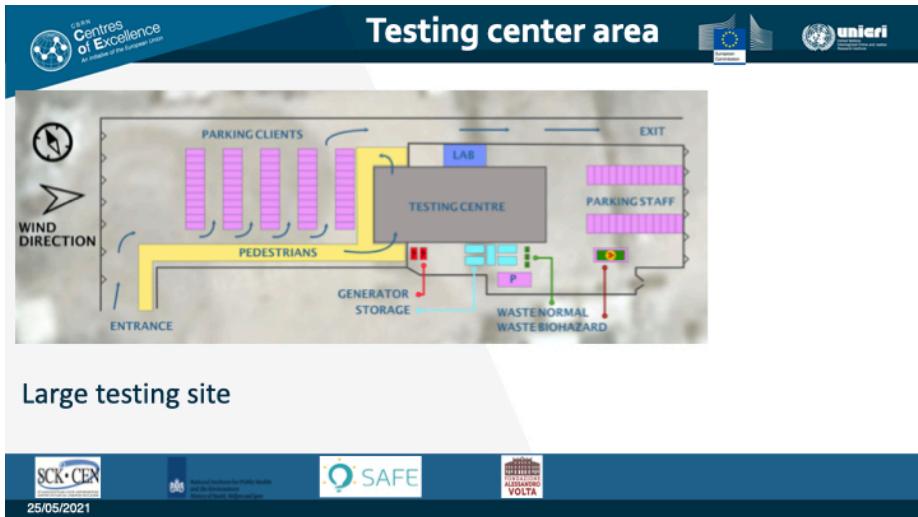


Figure 5.10: sheet 04

existing facilities, for example in a hospital. However, the large influx of visitors can cause problems, for example from traffic jams and large crowds.

It is therefore recommended for the testing area to set up in places that can be easily reached and has plenty of parking space. On the sketch the testing center is in between two areas. One for the clients and the other for staff. The areas can be separated by fences. Traffic controllers check at the entrance if visitors come for testing, and not because they want to park their car and go shopping at the nearby mall. The traffic controllers can also help prevent that the testing center gets overcrowded. Other things to consider when the testing site is set up, are canals to drain excess of water during heavy rain, or damage from heavy winds. Here is a picture of a testing-site in The Netherlands [PHOTO_04_1]. The entrance and exit are clearly marked. Also the Emergency exits need to be clearly visible. [PHOTO_04_2]

Some visitors may be handicapped or are less mobile. A dedicated parking space for immobile clients close to the entrance might be helpful.

Some of the waste should be considered as biohazard. [PHOTO_04_3]. The waste can be disclosed from the public and marked with a biohazard sign.

Sheet 05 - Small test center

Here is a sketch from a small test centers. These can be opened up at any location. It can even be a transformed cafeteria [PHOTO_05_1] or a camping van [PHOTO_05_2].

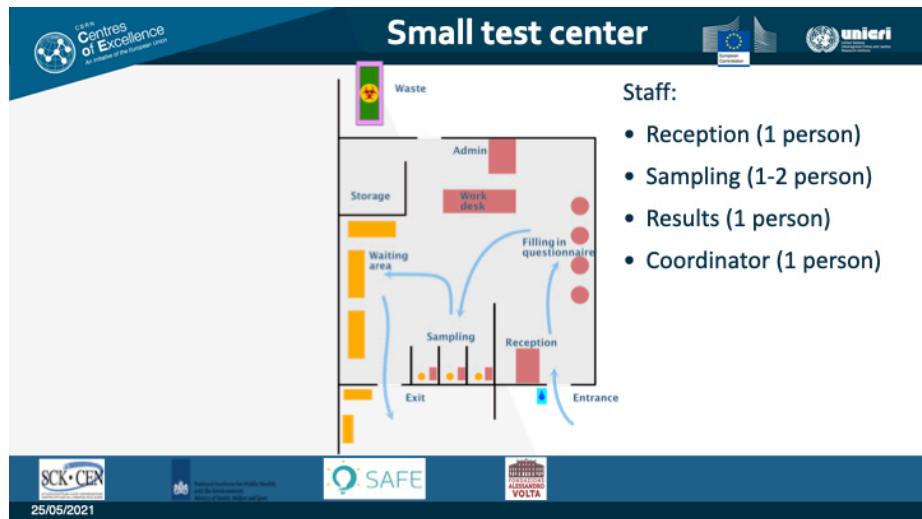


Figure 5.11: sheet 05

On the lay-out you can see that the clients all follow one direction. At the entrance the host asks if the client has specific symptoms and can check the temperature. The client receives a questionnaire [PHOTO_5_3], which might be translated into different languages. The clients can fill in the questionnaire separately at a table. Next, they hand over the paper to the sample taker. [PHOTO_05_4; PHOT0_05_5]. Another staff member receives the samples together with the client forms. This person makes sure that the results are read at 15 minutes, for example by using these cooking clocks [PHOTO_05_6]

The testing site can have an additional person who is responsible for the inventory and jumps in when extra help is needed. Furthermore, there should be a clear protocol for certain situations. What if the client has COVID-symptoms? What should be done when a test result is positive? What should be done when the quality control of the test fails? This is all part of the Quality Management of the site, which was discussed in the previous module.

Sheet 06 - Large test center I

Here, the lay-out of a large testing facility is drawn. The yellow part is the dedicated Clients area. The green part is the area for the staff and where clients are not allowed. Behind the testing-booths, there is a separate corridor. The staff can reach the testing booths from behind, and they don't have to cross the area with the clients. In the next slide I will go step by step at each location inside the testing center.

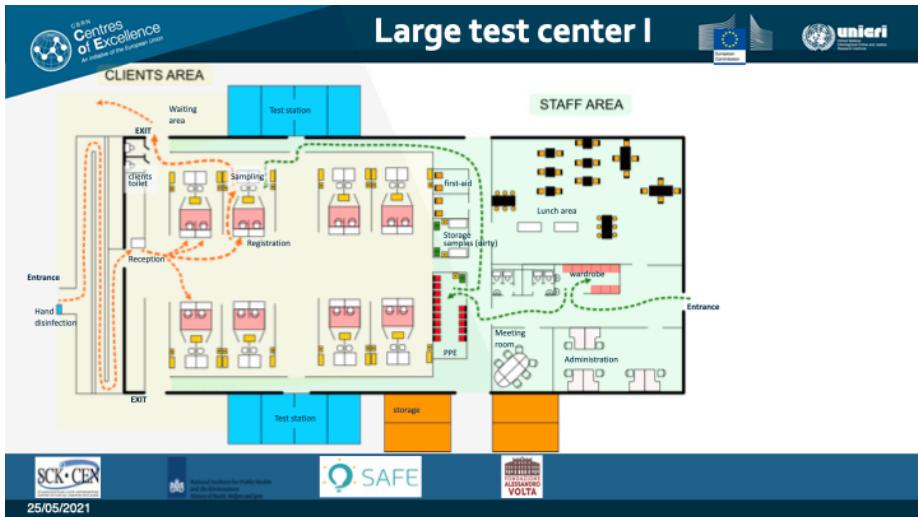


Figure 5.12: sheet 06

Sheet 07 - Large test center II

First the clients area. All the way at the left is the entrance, still outside the center. The client disinfects the hands and walks down the aisle. As you can see, the client has to make this detour to reach the reception. The detour takes around 30 seconds, exactly the time needed for the hand-disinfectant to do its job. The host at the reception can do the first intake. For example, to check if the clients has their identification with them. The host can provide instructions to go to an available booth. [PHOTO_07_1] If it concerns a child for example, they can be directed to a special booth that is arranged specially for children. Or direct someone to a booth that is accessible for their wheelchair. [PHOTO_07_2] The booths can have red or green light or monitor screens to indicate if the booth or lane is available.

Here is a photo of the registration counter. [PHOTO_07_3] There is glass in between. On the glass there is clearly indicated that the ID-card can be presented in the square on the glass. This to prevent that clients push their papers directly under the glass. Next, the client moves to sampling spot behind the booth. Here, attention should be paid that there is no mismatch between the client, and the information on the test-tube. There can be for example a centralized computer system where a sticker with a bar-code comes out of a label printer at the sampling spot. [PHOTO_07_4] Still, the person taking the sample should always double check if the information on the test-tube matches the information from the client.

When sampling is done, the client is directed to the exit. [PHOTO_07_5] Colored lines on the floor can help the client to reach the nearest exit. Outside,

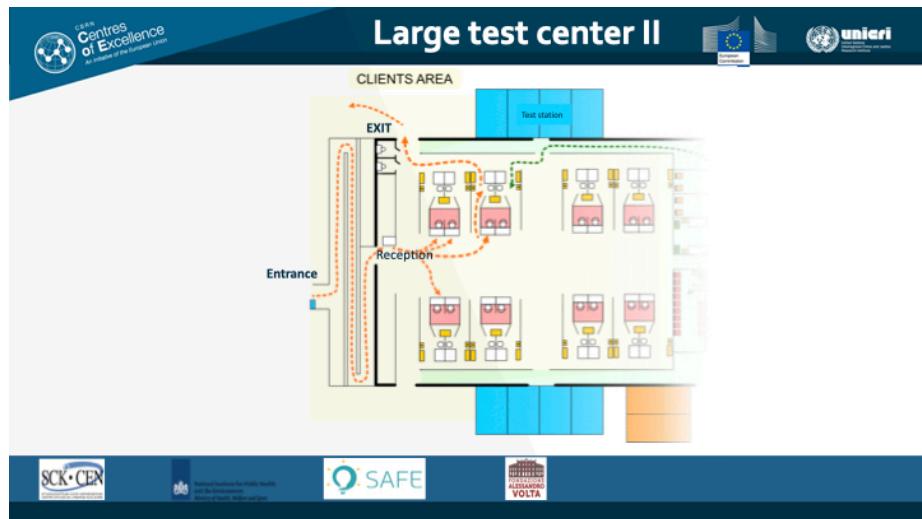


Figure 5.13: sheet 07

the client can wait for the test result.

Sheet 08 - Large test center III

Now we enter the testing facility from the staff entrance. The personnel can lock their properties in in the wardrobe. In the lunch area, before the shift starts, the personnel gets the latest updates and their work-sheets. After a coffee, the staff goes to the room where they put on their personal protective equipment or PPE. [PHOTO 08_1 & 08_2]. The tables should be clearly labeled that they are clean. Meaning, no used PPE are allowed on or around the table. This can also be highlighted with green and red tape.

For large testing sites, basic walkie talkies can be used [PHOTO_08_3] to stay in contact with colleagues, without the need of walking around with potential contaminated dressing and equipment, or shouting for assistance. Here is a pictures of two lanes separated with a wall [PHOTO_08_4]. The lane on the right is for staff. The left lane is for clients, with the blue line on the floor that directs them to the exit.

The staff member walks through the staff-corridor to their booth [PHOTO_08_5]. At the booth, the administrator takes seat behind the desk. If the place is well ventilated, he or she does not need full PPE. However, proper distance should be kept from the colleague behind who is taking swabs from clients.

The person taking swabs makes sure that there is plenty of material [PHOTO_08_06] before he or she starts the shift.

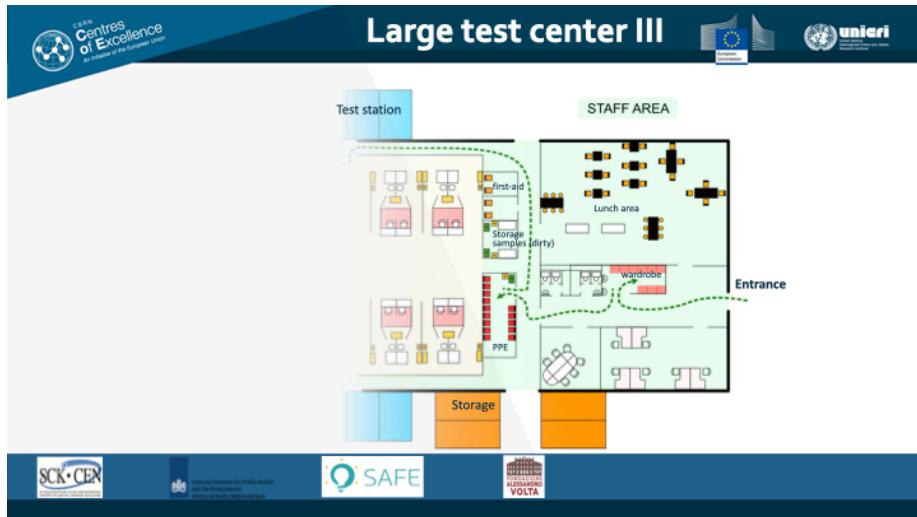


Figure 5.14: sheet 08

The tubes with the swabs can be collected and moved to the test station. There, they are tested with the Ag rapid tests or rapid PCR tests. [PHOTO_08_7] This picture is taken from a PCR laboratory, which needs an air-confined containment. Here, the samples are passed to the air-controlled laboratory through a lock with two windows. This strict air-containment is not needed when Antigen or serology tests are performed. If samples need to be send to a different location, they can then be stored in a refrigerator. [PHOTO_08_8] These can be for example samples collected for additional genotyping analysis for monitoring SARS-CoV-2 strains.

Sheet 09 - Large test center IV

Basically, at the test-center we need personnel for administration, we have a person taking the samples, a person collecting all the samples and moving them to the test station. We have someone dedicated to fill up the inventory. If a problem occurs on the floor, for example a mismatch with someones identification, a floor manager can jump in and provide assistance. Occasionally it can happen that someone gets a bleeding nose, or that someone faints. These clients can be helped in the first-aid room by someone who is trained for this.

Sheet 10 - Drive through

These are picture of a drive through testing site. It is the same principle, although clients do not get their test-result in person, but receive them by email or on an app. At the entrance the host directs the client to an available lane.

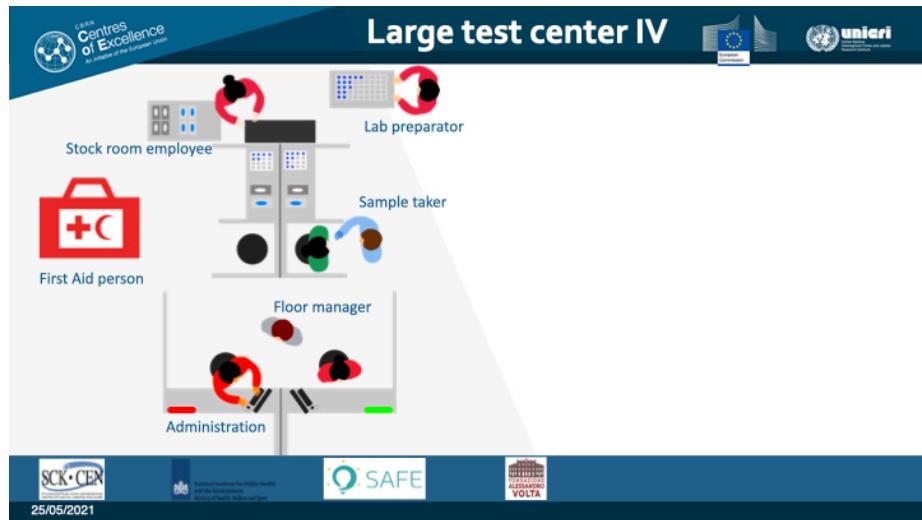


Figure 5.15: sheet 09

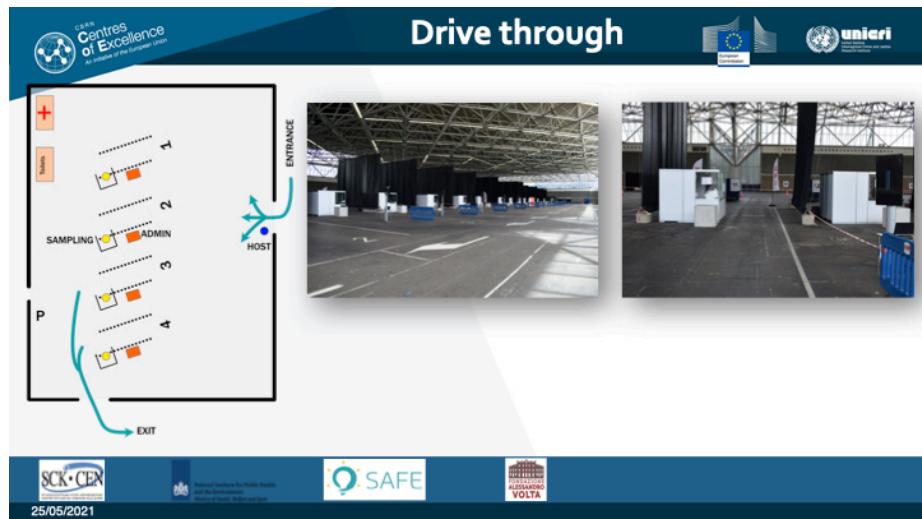
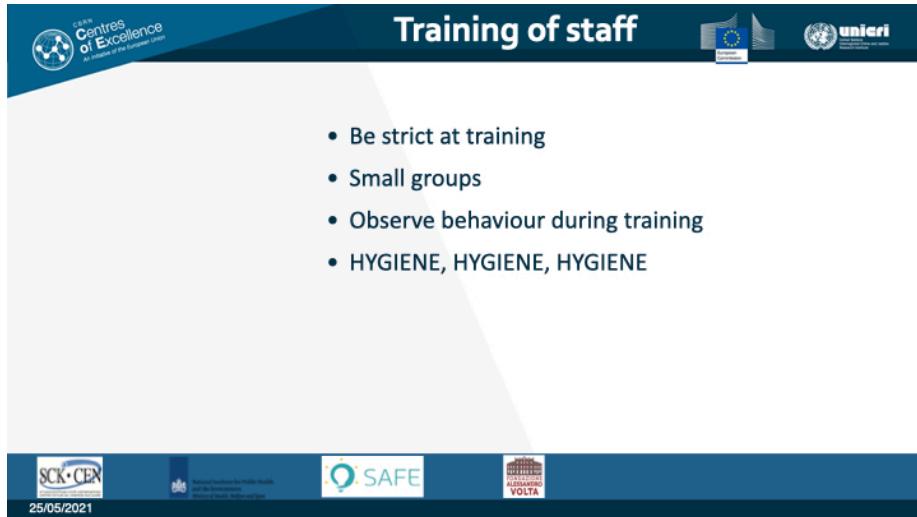


Figure 5.16: sheet 10

Engines should be turned off when the care is standing still to protect staff from car-fumes. There is a parking spot at the back if, for example there an administrative or medical problem, and assistance is needed from the floor manager.

Sheet 11 - Training of staff



- Be strict at training
- Small groups
- Observe behaviour during training
- HYGIENE, HYGIENE, HYGIENE

Figure 5.17: sheet 11

Some advice on training of new staff. [PHOTO_11_01]. Be strict at the training. If during the training the sample-taker is very anxious on taking swabs, don't push it and do not continue to train this person for this task. [PHOTO_11_02] Do the trainings in small groups, for example up to five persons. This for COVID safety reasons, but also to be able to observe the students closely. [PHOTO_11_03] If they don't follow the hygiene rules strictly, or show up late, or are impolite, then, at the end of the day, they should be asked not to continue. Personnel that are not reliable in their behavior and work ethics, they can put other staff members in danger. For the protection of clients and colleagues, the staff needs to be trained for a strict hygiene mindset.

Sheet 12 - Safe workspace

[PHOTO_12_1] Again, make sure all staff is well trained on hygiene and biosafety. For example, storage room for PPE is strictly off-limits for someone who was taking swabs. Or when receiving items from the clients. [PHOTO_12_2]. Security personnel might sometimes be needed at large testing sites. Clients can become frustrated and angry and attack the staff. Or to prevent theft. [PHOTO_12_3] At large testing sites, walkie talkies can be



Figure 5.18: sheet 12

useful, if they are used properly. Make sure staff know how to use them. Who do you need? But do not use full names. But for example, when you are the swab taker and you ran out of swabs, say: "Stock person for Swab-person at booth 5, over".

You can also use code words if it is for everyone and quick action needs to be taken. Code green for minor problems. Code orange incase of a more pressing issue, for example a client with a bleeding nose. And code red for all hands on deck, someone is attacking your colleague.

[PHOTO_12_4] For first aid, a separate space can be set-up.

[PHOTO_12_5] There should be copies of emergency procedures on all working areas. For example what to do in case of a biting incident.

[PHOTO_12_6] Emergency exits are clearly indicated. Fire extinguisher are regularly checked and smoke detectors are placed.

Sheet 13 - Tips & Tricks

[PHOTO_13_1] Set-up dedicated areas where staff and clients are separated as much as possible. For a fast through put, set-up a one-directional flow.

[PHOTO_13_2] Also, make everything as dummy-proof as possible. Both for the clients and for the staff. Make clear marks, which areas are clean and which areas are dirty/ or potentially contaminated. Also, everyone understands the definition of 'Clean' and 'Dirty'.

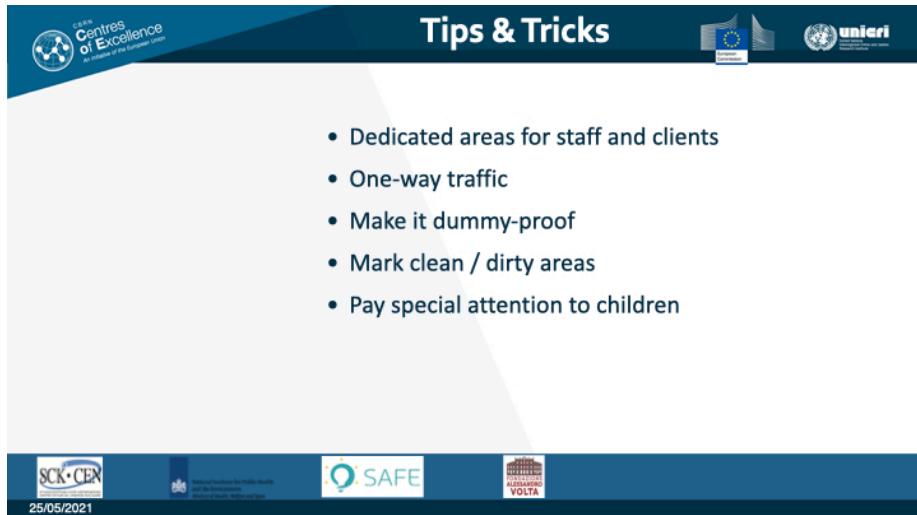


Figure 5.19: sheet 13

[PHOTO_13_3] Not only children may need special care and attention, also the parents may need to be addressed. Some parents might yell at their children, causing them to be even more distressed. Some staff members might be better in handling these kind of situations than others. Also, some rewards might be given to children. A diploma for bravery and some sweets.

Sheet 14 - Summary

There was an overview of the types of tests that are being applied for detecting COVID. It is made clear that one cannot just simply compare one test with another. Especially when they are applied in different settings. Module three explained how to validate a new test for a large scale testing program. Module 4, explained about continuous monitoring and quality assurance of a testing site. And in this module, the set-up of a large and small testing sites was presented.

5.5.3 In-depth questions

5.5.3.1 Question 1

Left picture: Stock items should be clean. The lab preparator carries potentially contaminated samples. The desk of the sample taker is exposed to potential COVID-19 patients. The administration should be shielded from clients and sample-taking area to keep it 'clean.'

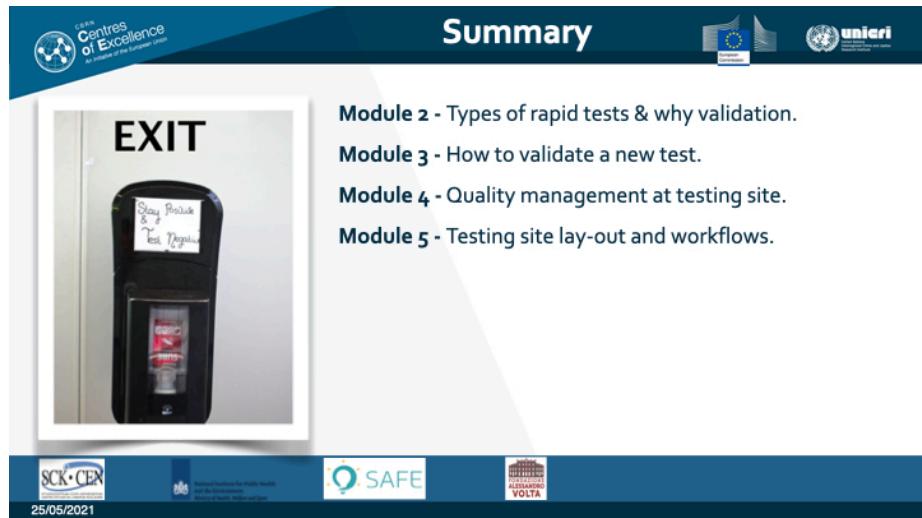


Figure 5.20: sheet 14

The slide has a blue header with 'Centres of Excellence' and 'An initiative of the European Union'. The title 'Question 1' is centered above two images: a schematic diagram of a laboratory layout and a photograph of a person in a lab coat. The schematic shows 'Stock room employee', 'Lab preparator', 'Sample taker', and 'Administration' areas with question marks indicating contamination risk. The photograph shows a person in a lab coat standing near a counter with a 'WalkieTalkie' device. To the right, text reads: 'Should these spots be considered 'CLEAN' or 'CONTAMINATED'?'. Logos for SCK-CEN, National Institute for Public Health and the Environment, Istituto Superiore di Sanità, and Istituto Alessandro Volta are at the bottom, along with the date '04/05/2021'.

Figure 5.21: question 1

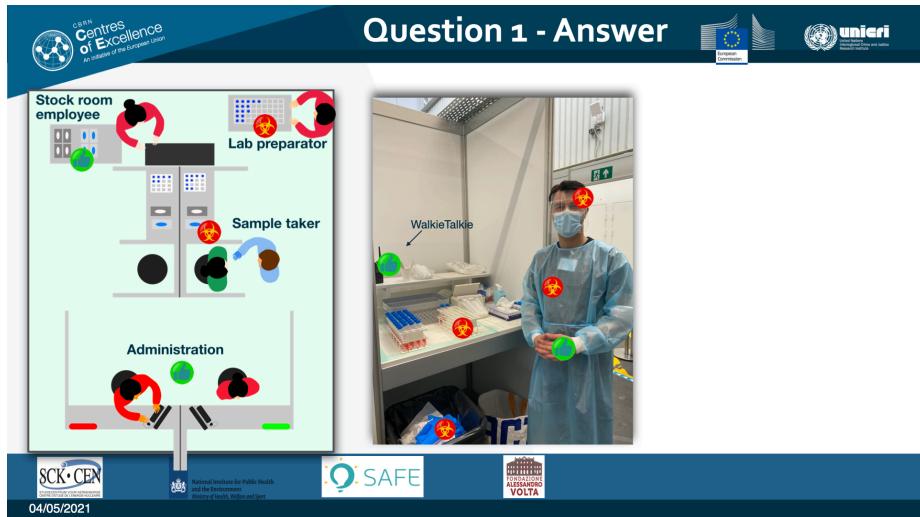


Figure 5.22: answer 1

Right picture: The sample taker is not wearing gloves. The hands should be clean if the gloves were removed properly. Only then, (s)he can pick up the 'clean' walkie talkie. All the rest should be considered as potentially contaminated or 'dirty.'

5.5.3.2 Question 2

Question 2

The slide features two photographs on the left: one of an office desk with a chair and a power cord on the floor, and another of a storage area with boxes and containers. On the right is a flowchart titled "Sampling" showing a circular process between "Sampling", "Filling in questionnaire", "Direction for clients", "Reception", "Work desk", "Storage", "Waste", "Sampling", and "Waiting area". Logos for CEN, unicef, SCK-CEN, and the National Institute for Public Health and the Environment are at the bottom.

Safe workplace?

**What are the risks
and how to
improve?**

04/05/2021

Figure 5.23: question 2

Question 2 - Answer

The slide includes the same photographs and flowchart as Figure 5.23. It adds several callout boxes with risk assessments:

- Risk of tripping over cables. Damage to cables can cause short circuit. Guide cables along the wall.
- Carton boxes on floor. Risk of mould and water damage to material. Contents not visible. Use pallets & cabinets.
- Sampling areas not divided.
- Clients and staff are in each other's way in the room. Risk of contamination.
- No one-directional flow for clients.
- In case of emergency situation, only one exit.
- Public access to waste and storage. No easy/safe access for staff.

Logos for CEN, unicef, SCK-CEN, and the National Institute for Public Health and the Environment are at the bottom.

04/05/2021

Figure 5.24: answer 2

Chapter 6

Practical trainings

This chapter lists several practical exercises that can be conducted in groups of 5 to 10 persons. The exercises can be alternated with the theoretical presentations listed in chapter Presentations.

6.1 Introduction to Practical training

Length	10-20 minutes
Learning goals	Participants explain what they want to gain from the training. Participants can explain that good procedures and good test material are equally important.
Summary	Introducing by name and position. Discuss the goal of the training. Gain knowledge about types of tests and importance of good procedures.
Setup	The trainer explains the purpose and the outline of the training. The participants explain why they are at the training and what they wish to retrieve from the training The trainer makes notes of the participants responses on motivation of doing the training.
Material	<ul style="list-style-type: none">• two flip charts with easel or whiteboard• laptop computer• projector compatible with computer• extension cord• wastebasket• markers• note pads (one per participant)• pens and pencils (one per participant).

6.2 Donning & Doffing

Length	1 hour
Learning goals	Participants can: <ul style="list-style-type: none"> • put on and remove PPE appropriately
Summary	Prevent Covid-19 droplet contamination by the appropriately putting on, and removing PPE. The procedure is demonstrated first by the trainer. The procedure is repeated by one or two participants. Optional: a UV-lamp and fluorescent gel can be applied on the participants hand to check for contamination.
Setup	Dressing procedures are: <ol style="list-style-type: none"> 1. Removing jewelry, watch, etc. Hair is tight back. 2. Perform hand hygiene 3. Putting on the gown 4. Putting on the mask 5. Putting on eye protection 6. Putting on gloves Taking off dressing procedures are: <ol style="list-style-type: none"> 1. Removing gloves 2. Removing gown 3. Removing eye protection 4. Removing mask 5. Performing hand-hygiene Evaluate appropriate dressing afterwards. Ask questions such as: Why closing the gown at the back? Why remove mask as last? Optional: Check for contamination with UV-lamp. <ul style="list-style-type: none"> • Hand disinfectant • Gowns, at least three per group • Masks, at least three per group • Face protection, at least three per group • Gloves, 1 box of each size. Optional: <ul style="list-style-type: none"> • Fluorescent gel to be put on gloves, and UV-lamp.

6.3 Sample taking & rapid test

Length	1 hour and 30 minutes
Learning goals	Participants can: <ul style="list-style-type: none"> • Take nasal and oropharyngeal swab • Perform rapid diagnostic antigen test
Summary	Practical on performing the test procedure.

Setup	<p>Participants watch video: collect oro_nasopharyngel_specimens_COVID19.mp4</p> <p>Group is split with 5(?) persons per group. Each group has volunteers to: 1) get tested with the swabs, 2) perform the two swabs taking, 3) perform the rapid test following the package instructions.</p> <p>Swab-takers do hand hygiene and put on full PPE. Testers do hand-hygiene and put on gloves.</p> <p>If positive control is available in the kit, use it for showing to participants.</p> <p>Note: be prepared in case participant appears positive. Test result can become false-positive when left on table too long. Be also prepared that participant can get bleeding nose.</p>
Material	<ul style="list-style-type: none"> • 1x PPE for each group • Gloves • 1x rapid diagnostic test kit for each group • Chair & table for each group • Disinfectant for hands and for material • tissues

6.4 Standard Operation Procedures

Length	1 hour
Learning goals	<p>Participants can:</p> <ul style="list-style-type: none"> • explain importance of clear procedures • argue difficulties in test procedures • recognize critical parts during the procedure • solve incidents during testing
Summary	Importance of good standard procedures and to be better prepared for unsuspected incidents.
Setup	<p>Participants watch video: Instructions_Peanut_butter_jelly.mp4</p> <p>and/or</p> <p>Do exercise Instructions_Paper_folding.pdf</p> <p>Group discussion. Trainer can ask what if questions such as:</p> <ul style="list-style-type: none"> • What do you do if client faints/gets bleeding nose? • What to do when control-line of test is not visible? • For watching video • A4 papers for exercise instructions paper folding.
Material	

6.5 Root Cause Analysis

Length	1 hour
Learning goals	Participants can: <ul style="list-style-type: none"> • apply a root-cause analysis • predict bottlenecks and critical components of a testing facility.
Summary	Participants perform and discuss risks assessments, as part of continues quality assurance on the workfloor.
Setup	This practical should be done after the Quality Management presentation of module 4 . Small groups are formed. Each group prepares a Risk assessment example. Each group presents their Risk assessment.
Material	<ul style="list-style-type: none"> • Risk_assessment.docx, one print (A3) for each group.

6.6 Design test site

Length	1 hour
Learning goals	Participants can: <ul style="list-style-type: none"> • sketch a testing site lay-out • differentiate clean- and dirty zones
Summary	The participants work together with minimal support from the teacher to design a test site. The test site should be a small scale testing facility for the simulation in the training room.
Setup	Participants can take a role for each part in the design, e.g. logistics, administration, lab, crowd-control, swab-taking...
Material	This practical should be done after the Workflow presentation of module 5 . Trainer explains definitions of 'Dirty' and 'Clean'. Trainer explains that participants need to set-up and simulate a test-center according to the design they are preparing. <ul style="list-style-type: none"> • Flip-over • Marker pens, at least three different colors • Beamer, screen, computer & speaker

6.7 Setup test site

Length	45 minutes
Learning goals	Participants can: <ul style="list-style-type: none">• Create a testing facility taking into account the essential standards for a safe and high-throughput testing-flow.
Summary	This practical follows the ‘presentation on Workflow’ and the ‘practical on Design test site.’ The participants work together with minimal support from the teacher to set-up the test site.
Setup	Ideally, the test-center has a separate entrance and exit. Participants use their design and the material to furnish the test-center. The trainer should intervene as little as possible as the participants might encounter flaws in the design by themselves during the simulation. Assign roles for participants: Host, administrator, sample-taker, clients, observer (quality manager). Each participant chooses a personage and fills in the personality traits. For example, a bossy floor manager, an aggressive client, a clumsy sample-taker. The auditor should take more the role of the observer.

Material	<ul style="list-style-type: none"> • Tables (~6) • Chairs (~4) • Tape for fencing the areas and walk-directions (at least three different colors) • new (unopened) sterile swabs for each participant to perform three sample collections (these may be sold separately and must be compatible with the test kit, or they will be included in the standard test kit contents) • personal protective equipment (PPE), including gloves, gowns, eye protection or face-shields, respirators (N95 or FFP2) (various sizes), and medical masks • pens for marking or labelling • household bleach (3–5%), ethanol (70%) and paper towels to clean the workstation and hands • soap for hand-washing or alcohol-based hand gel • sufficient test kits for each participant to perform tests • leak-proof biohazard bags for containing or moving biohazard waste (1-2) • waste bins for biohazard bags (2) • two spray bottles (one for bleach, the other for ethanol) per workstation • timers (5) • Registration sheet (registration.docx) • Questionnaires (questionnaire.docx) • Writing pads. • Print-outs of the profiles • Standard operation procedures & Instructions
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6.8 Simulate test site

Length	1 hour and 30 minutes
Learning goals	<p>Participants can:</p> <ul style="list-style-type: none"> • Create a testing facility taking into account the essential standards for a safe and high-throughput testing-flow.
Summary	<p>The participants selected a role for themselves and prepared their particular character traits.</p> <p>The testing facility is then simulated and dramatised.</p>

Setup	<p>This practical follows the ‘presentation on Workflow’ and the ‘practicals on Design- and setup the test site.’</p> <p>Performance of all participants. Participants pick a role and fill in their character traits. Trainer explains that it is ok to exaggerate in their role to see how other react.</p> <p>After the simulation, all participants reflect on the simulation. What went well? What could be improved?</p> <p>How would others handle certain situations?</p>
Material	<ul style="list-style-type: none">• Tables (~6)• Chairs (~4)• Tape for fencing the areas and walk-directions (at least three different colors)• new (unopened) sterile swabs for each participant to perform three sample collections (these may be sold separately and must be compatible with the test kit, or they will be included in the standard test kit contents)• personal protective equipment (PPE), including gloves, gowns, eye protection or face-shields, respirators (N95 or FFP2) (various sizes), and medical masks• pens for marking or labelling• household bleach (3–5%), ethanol (70%) and paper towels to clean the workstation and hands• soap for hand-washing or alcohol-based hand gel• sufficient test kits for each participant to perform tests• leak-proof biohazard bags for containing or moving biohazard waste (1-2)• waste bins for biohazard bags (2)• two spray bottles (one for bleach, the other for ethanol) per workstation• timers (5)• Registration sheet (registration.docx)• Questionnaires (questionnaire.docx)• Writing pads.• Print-outs of the profiles• Standard operation procedures & Instructions

Appendix I - list materials

6.9 Material, supplies and kits for demonstration and practical

The following items are required for the practical training:

- Tables (~6)
- Chairs (~4)
- two flip charts with easel or whiteboard
- laptop computer
- projector compatible with computer
- extension cord
- wastebasket
- markers, at least three different colors
- note pads (one per participant)
- pens and pencils (one per participant)
- Tape for fencing the areas and walk-directions (at least three different colors)
- new (unopened) sterile swabs for each participant to perform three sample collections (these may be sold separately and must be compatible with the test kit, or they will be included in the standard test kit contents)
- personal protective equipment (PPE), including
- gloves, gowns, eye protection or face-shields, respirators (N95 or FFP2) (various sizes), and medical masks
- pens for marking or labelling
- household bleach (3–5%), ethanol (70%) and paper towels to clean the workstation and hands
- soap for hand-washing or alcohol-based hand gel
- sufficient test kits for each participant to perform tests
- leak-proof biohazard bags for containing or moving biohazard waste (1-2)
- waste bins for biohazard bags (2)
- two spray bottles (one for bleach, the other for ethanol) per workstation
- timers (5)
- Mod4_quality.mp4

- Mod_5_workflow.mp4
- Peanut_butter_instruc.mp4
- Paper_folding_instruc.pdf
- Registration sheet (registration.docx)
- Questionnaires (questionnaire.docx)
- Risk_assessment.docx, one print (A3) for each group

Chapter 7

Frequently Asked Questions

This chapter lists examples that can be asked by participants during the training.

7.1 Definitions

Q: What is the difference between SARS-CoV-2 and COVID-19?

A: SARS-CoV-2 is the abbreviation for SARS coronavirus and the cause of severe acute respiratory distress syndrome.

COVID-19 stand for **COrona VIrus Disease 2019**. It is a contagious viral disease caused by SARS-CoV-2.

Q: What is the difference between PCR, RT-PCR and LAMP-PCR?

A: - PCR is the abbreviation for Polymerase Chain Reaction. It is a technique to multiply a small piece of DNA to an extend that it can be detected.

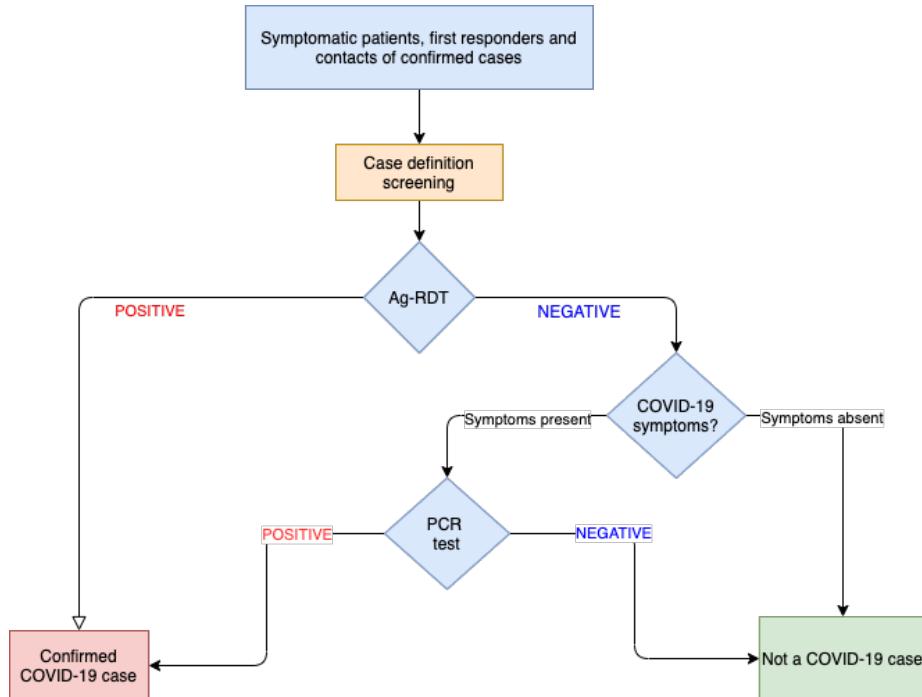
- RT-PCR stands for Reverse Transcription PCR. An additional reaction step is added prior to the PCR step, where RNA is first transcribed into DNA. Hepatitis B virus is a ‘DNA-virus’ and can be detected directly with PCR. SARS-CoV-2 is an ‘RNA-virus’ and needs RT-PCR for detection.

- LAMP stands for Loop-mediated isothermal amplification reaction. It is a single-tube technique for the amplification of DNA. It can be combined with the reverse transcription step as a low-cost alternative to detect SARS-CoV-2.

7.2 Algorithm

Q: What is the testing algorithm for diagnosing COVID-19?

A: The testing algorithm depends on the setting and available resources. For example, in a setting with limited access to PCR-testing, the testing algorithm can be described as in the scheme below:



Q: What if the testing outcome is negative, but SARS-CoV-2 infection is still strongly suspected?

A: An decision tree can be extended for specific persons, such as those tested negative but with strong suspicion of COVID-19. For these cases, testing can be repeated 3-5 days after the first test.

Q: What is the rationale for deciding to repeat the Ag-RDT / PCR test after 3-5 days?

A: The viral load can be too low shortly after exposure. Since Ag RDT detects the presence viral proteins, it is more likely to detect the virus at a later, infectious state.

Q: When should an Ag-RDT be applied after high risk contact? eg. 48 or 72 hours after high-risk contact?

A: The median incubation period for SARS-CoV-2 is 4-5 days so it's best to test 4-5 days after contact. It is not a requirement to test all contacts as resources may be limited. Better to focus on those at higher risk of SARS-CoV-2. A negative test doesn't shorten quarantine - but a positive case allows you to rapidly pursue secondary contact tracing.

Q: What is the relevance of testing asymptomatic persons with Ag-RDTs?

A: Asymptomatic individuals infected with SARS-CoV-2 can transmit infection. However, most transmissions are from symptomatic individuals and that is why rapidly identifying symptomatic persons and implementing control measures should be the first priority. Asymptomatic contacts of cases and individuals who are frequently exposed such as health workers, are a second priority.

7.3 Testing

Q: What are some limitations in the use of Ag-RDTs?

A: The major limitation of Ag-RDTs is the requirement to have a high antigen load in the body at the time of testing. This means that the tests are most accurate for symptomatic patients (with a higher antigen load) within the first week after onset of symptoms. The tests are less sensitive to detect asymptomatic people.

Q: What are the possible causes of a Positive Ag-RDT but Negative in PCR?

A: This could be a false positive Ag-RDT, or a false negative PCR (eg. it failed, for example there was an inhibitor in the reaction). It would be best to repeat the sample to decide whether it is SARS-CoV-2 positive or negative.

Q: Why do Ag-RDTs have high false negative results (low sensitivity)?

A: Ag-RDTs rely on the presence of virus-antigen in the sample. If there is low antigen, for example because of to the stage of the illness, or mistakes during the sample collection or low sample-quality, then the test may be mistakenly negative, when in fact the person carries SARS-CoV-2. A PCR test is able to better detect infections as it involves amplifying the small amount of SARS-CoV-2 in the sample. Impact of SARS-CoV-2 variants on Ag-RDT performance should

also be considered as a cause for false negative results. There are, however, very few cases described.

Q: What is the best method to send an Ag-RDT sample to the lab for confirmation?

A: For the laboratory to check for SARS-CoV-2 with another test, for example PCR, they will need a sample directly from the body - eg. a swab, or the eluate from a swab. The lab won't be able to use the first Ag-RDT swab sample to re-test.

Q: Can samples be pooled for SARS-CoV-2 Ag RDTs?

A: Any tests should be checked that they are used as recommended by the manufacturer. Any changes to the protocol requires validation. In any case, the more samples used/pooled, the more the sensitivity will decrease. In some countries, samples are pooled for RT-PCR.

Q: Can saliva (spit) be used for Ag-RDT?

A: There are several studies where Ag-RDTs were used on samples which were NOT recommended by the manufacturer, for example comparing nasopharyngeal swabs with saliva. The sensitivity usually is lower for the 'new type of samples.' Still, some manufacturers validated their Ag-RDT for use with saliva, although they still appear to have much lower sensitivity. New tests may be developed in the future.

Q: Are Ag-RDT kits validated for different variants?

A: The WHO is tracking reports for variants that may slip through diagnostics. So far, there is no evidence of specific SARS-CoV-2 variants not being able to be detected with (specific) Ag-RDTs. As new variants emerge, manufacturers are assessing the performance of their tests. In addition, independent research groups are also conducting assessments

Q: Will Ag-RDT and/or PCR test become false positive after Adenovirus- and mRNA-based vaccinations?

A: Vaccination against SARS-CoV-2 will not result in a positive diagnostic test. Vaccines do NOT replicate inside our body to make high levels of antigen and so, it would not be detected by the Ag-RDTs. The mRNA vaccines induce cells to make antigens, still, it is much lower than by a natural infection. In addition, there is no/limited overlap in the virus proteins targeted by the vaccines and the

Ag-RDTs. AgRDTs should still be able to detect true SARS-CoV-2 infection. More information on vaccination and testing accuracy can be found on this website.
