Tests for diagnostics of COVID-19 - principles and approvals of commercially available tests

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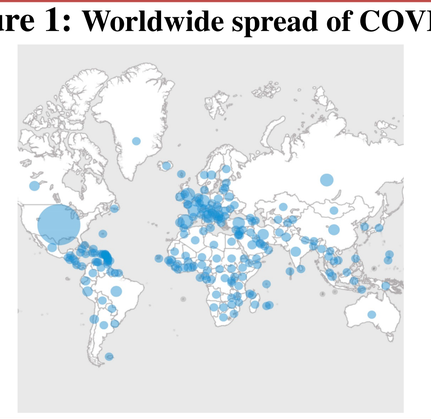
COVID-19 disease

In December 2019 an unknown viral infection was first described in a local fish and wild animal market in Wuhan/China which was identified as a novel coronavirus infection by the Chinese Center for Disease Control and Prevention (CCDC) on Jan. 7th 2020 and announced as 2019-new coronavirus disease (2019-nCoV, now COVID-19) by the World Health Organization (WHO) on Feb. 11th 2020. Rapidly spreading across the globe by May 11th 2021 at least 158.7 million of infections and 3.3 million deaths were reported worldwide (**Fig. 1**). However, large differences in respect to distribution and lethality were reported between different countries (**Figs. 1 and 2**). In the initial stages of the disease the virus affects the upper respiratory tract and some time later, the lung, causing severe respiratory symptoms. The spread of the infection is predominantly caused by the production of infectious aerosols distributed by expired air. However, the virus causes also systemic symptoms, affecting vascular system and solid organs causing thrombosis, strokes and organ failure. Due to the rapid spread of the disease there was an urgent need for in-vitro diagnostic tests for diagnostics. However, the type of developed tests changed within the course of pandemic.

Figure 1: Worldwide spread of COVID-19 disease.



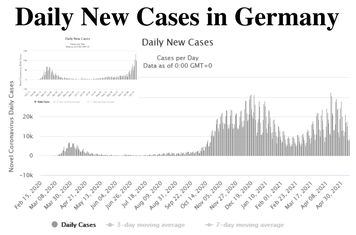
Globally, as of 7:07pm CEST, 11 May 2021, there have been 158.651.638 confirmed cases of COVID-19, including 3.299.764 deaths, reported to WHO. As of 10 May 2021 a total of 1.206.243.409 vaccine doses have been administered. https://covid19.who.int/

Cases by country		Deaths by country	
US	32.778.621	US	582.845
India	23.340.938	Brazil	425.540
Brazil	15.282.705	India	254.197
France	5.861.384	Mexico	219.323
Turkey	5.059.433	UK	127.890
Russia	4.840.948	Italy	123.282
UK	4.455.446	Russia	112.063
Italy	4.123.230	France	107.096
Spain	3.586.333	Germany	85.385
Germany	3.557.904	Spain	79.100
Argentina	3.191.097	Colombia	78.771

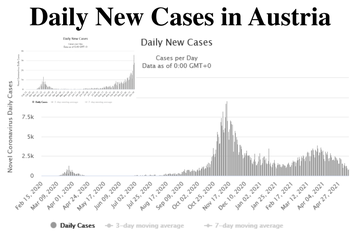
Date: May 12th https://coronavirus.jhu.edu/map.html

Figure 2: COVID-19 disease in selected countries.

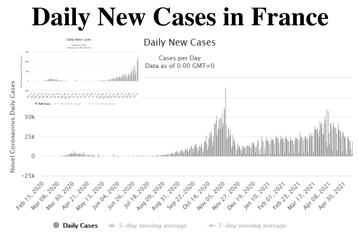
Daily New Cases in Germany



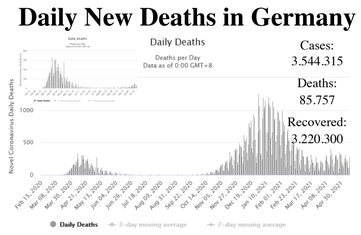
Daily New Cases in Austria



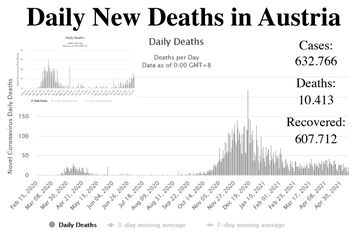
Daily New Cases in France



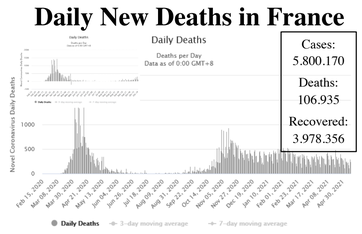
Daily New Deaths in Germany



Daily New Deaths in Austria



Daily New Deaths in France



Date: May 12th https://www.worldometers.info/coronavirus/country/germany/

Date: May 12th https://www.worldometers.info/coronavirus/country/austria/

Date: May 12th https://www.worldometers.info/coronavirus/country/france/

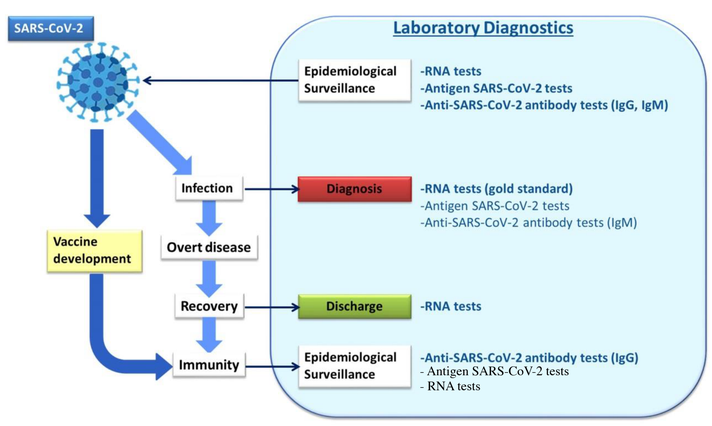
In-vitro diagnostic tests – legal requirements

In-vitro diagnostic tests (IVD) including those for diagnostics of COVID-19 are regulated by Directive 98/79/EC on *in vitro* diagnostic devices (the IVDD). As of 26th May 2022, the Directive will be replaced by Regulation (EU) 2017/746 on *in vitro* diagnostic devices (IVDR). When assessing conformity with the legislation and prior to affixing the CE-mark, the manufacturer must evaluate the performance of the device and report the performance information in the instructions for use and technical documentation of the device. This is usually achieved by conducting performance studies. In addition to this, after market introduction performance of devices may be validated, i.e. confirmed by additional testing that the manufacturer's specifications are indeed satisfied, e. g. in reference laboratories, academic institutions or national regulatory agencies. Such validation is not legally obligatory but highly recommended for public health decision making, especially in the context of the current COVID-19 crisis. Validation can be done not only for CE-marked devices but should also be performed for in-house laboratory protocols. In contrast to the European regulations IVD and medical devices in the United States need an approval of the Food and Drug Administration (FDA) prior to marketing. As there was urgent need for diagnostic tests at the begin (antibody tests, PCR tests) and later (antigen tests, self-tests) of the epidemic IVD for COVID-19 diagnostics were introduced into the European and US market after national time restricted approvals ("Sonderzulassung", duration 3 months) and Emergency Use Authorization (EUA), respectively.

Test principles

Depending on their diagnostic purpose (e. g. diagnostics of acute disease, diagnostics at the time point of discharge, epidemiological surveillance) tests of different analytical principles are used differing strongly in respect to their analyte (throat swab vs. blood/serum/plasma) as well as sensitivity and specifity. Tests are based on molecular means (RNA/PCR tests), antigen tests for detection of viral antigens and antibody tests (serological tests) for detection of IgM and/or IgG (**Fig. 3**).

Fig. 3: Testing in the context of COVID-19 disease.



Current performance of COVID-19 test methods and devices and proposed performance criteria - Working document of Commission services. Datum des Dokuments: 16/04/2020 - Erstellt von GROW.R.2.DIR - Datum der Veröffentlichung: n/a - Letzte Aktualisierung: 17/04/2020. https://ec.europa.eu/docsroom/documents/40805?locale=de (modified by authors).

Types of tests

Molecular tests: A large number of real-time PCR (RT-PCR) protocols to detect the presence of SARS-CoV-2 RNA have been published. Depending on the type of the used primers the method shows *in vitro* a very low limit of detection (LOD) with viral RNA transcripts (e. g. 11.2 RNA copies/reaction). RT-PCR methods require specific laboratory equipment and various sets of reagents and are performed by trained laboratory personnel proficient in performing the methods. A modification, digital PCR (dPCR) showed an improved lower limit of detection, sensitivity and accuracy compared to real-time PCR for low viral load diagnosis resulting in reduced false negative detection in samples from the lower respiratory tract, especially in cases of low viral load samples. However, this technique requires more sophisticated instruments. Few tests are based on CRISPR technology, widely known for its use in gene editing techniques, but in recent years it has been also used for the *in vitro* detection of nucleic acids, thereby emerging as a powerful technology for molecular diagnostics recognizing sequences of DNA, e. g. in viral genetic material. Few other techniques are based on LAMP, an isothermal variation of PCR (meaning it does not require dedicated and expensive thermal cycling equipment) and is therefore commonly used for point-of-care testing (POCT) due to its high sensitivity, rapid reaction and simple operation. The result is evaluated by a colour change that does not require specialized personnel (**Table 1**). A minor number of these tests allows detection/differentiation of disease, e. g. COVID-19/influenza.

Antigen tests: There are two major types of immunological tests for antigen detection, namely ELISA (enzyme-linked immunosorbent assay) and related principles as well as LFA (lateral flow assays). ELISA tests are more complex and require trained technicians operating under sterile laboratory conditions, but tend to be more sensitive. LFA are point-of-care formats (including patient self tests, nose/throat swabs, "lolly"-tests) which were developed with simplicity and portability in mind. They can be simple and easy-to-produce devices. For antigen tests the diagnostic sensitivity (proportion of those individuals with the target condition (infected individuals with reference SARS-CoV-2 RNA true positive specimen) who test positive with the antigen test) is the most crucial parameter for the correct identification of persons who are infected, as false negative test results must be as low as possible. The number of antigen tests in the market is rapidly increasing due to political reasons (testing for being "COVID-19 free" prior to entering shops or making holidays, testing in kindergardens/schools and industry), the lower price and more rapid results than PCR tests although analytical sensitivity tends to be lower than PCR. In addition, some tests allow determination of further antigens, e. g. influenza (**Table 1**).

Antibody tests: Most tests are targeting the immunoglobulins IgG and IgM (combined), fewer tests IgM or IgG and only very few tests IgA in blood/plasma/serum. They were designed to display the presence/absence of one of the two immunoglobulins or of both in one single test and provide rapid information about a past infection and immunological status. Tests using the virus spike protein to capture the antibodies seem to be more sensitive than the ones using the nucleocapsid protein. Tests that detect both IgG and IgM at the same time are superior to the ones testing for only one antibody depending on the kinetics of antibody production (IgM/IgG) after infection (increasing sensitivity between 7-14 days after infection) and as expected the results differ from those of PCR tests and antigen tests (**Figure 3**). Overall, ELISA-type (laboratory) tests seem to provide better results than flow-strip tests (LFA). Although the latter are faster and potentially suitable for point-of-care, the former seem to be more sensitive and reliable. However, more specific information is provided by a minor number of laboratory tests for neutralizing antibodies (3-5 % of circulating immunoglobines) introduced into the market in later 2020 (**Table 1**).

Table 1: Devices/tests listed in 3 selected data bases.

Sampling devices and tests. https://www.360dx.com/coronavirus-test-tracker-launched-covid-19-tests (download April 30th 2021)

	Total	U.S. Reg. Status	Eur. Reg. Status	Asia Reg. Status
All collection devices	17	16	1	0
Collection devices	7	6	1	0
Collection kits	6	6	0	0
Home collection kits	4	4	0	0
All tests	538			
Molecular based tests	36	27	10	3
PCR	328	248	118	21
Isotherm. Amplification	20	13	8	1
Sequencing	7	7	0	0
CRISPR	3	3	0	0
LAMP	2	2	0	1
LAMP sequencing	1	0	1	0
PCR microarray	2	1	1	1
PCR + mass spectrometry	1	1	0	0
Immunologically based tests	169	112	74	10
Serological	122	85	53	5
Antigen	47	27	21	5

Rapid tests for virus antigen detection listed by BfArM. Total number of listed tests: 444.

https://antigentest.bfarm.de/ords/f?p=110:100:3750320171253:::::&tz=2:00 (download May 11th 2021)

Note: list provides data of distributors, manufacturers, sensitivity, specifity, Instrucction for use (IFU) country and authorized representatives

	Number		Number
Test evaluated by PEI	148	Test not evaluated by PEI	296
POC without analyzer	134	POC without analyzer	270
POC with analyzer	8	POC with analyzer	20
POC with analyzer/laboratory	1	POC with analyzer/laboratory	0
No information	5	No information	6

Self tests (lay use tests) for virus antigen detection listed by BfArM. Total number of tests: 56.

https://antigentest.bfarm.de/ords/f?p=ANTIGENTESTS-AUF-SARS-COV-2:TESTS-ZUR-EIGENANWENDUNG-DURCH-LAIEN:15723270191005:::::&tz=2:00 (download: May 11th 2021)

Sample material	Number	Sample material	Number
Nasal	41	Saliva/nasal	1
Saliva	10	Saliva/sputum	2
Sputum	1	Saliva/sputum/nasal	1

Conclusion: An increasing number of tests, at latest antigen tests (LFA for professional/lay use) was introduced into the market. Described lists provide valuable data for tests in the market.