Spindiag

Rhonda

Rhonda SARS-CoV-2 disk

For scientific staff
SD003-02-I02-A02-EN

Analytical Principles

1 Information on this publication

Publication	Version	Changes
22 January 2021	SD003-02-I02-A02-EN	Initial publication in English.

1.1 User information

Please read these Instructions for use before working with the Rhonda SARS-CoV-2 disk.

Keep the Instructions for Use in a safe, easily accessible place.

We would like our language to be as inclusive as possible. We have therefore used neutral formulations wherever possible.

The user information for the Rhonda player comprises:

- *Rhonda player – Instructions for Use*
- *Rhonda player – Network and Service Documentation*

The user information for the Rhonda SARS-CoV-2 disk comprises:

- *Rhonda SARS-CoV-2 disk – Instructions for Use* *Instructions for Use* can be found on the side of every set of 20 Rhonda SARS-CoV-2 disks.
- *Rhonda SARS-CoV-2 disk – Analytical Principles*

1.2 Obtaining electronic copies

You can obtain electronic copies of all the Instructions for Use from the Spindiag website. All electronic Instructions for Use are available in a number of different languages.

Tablets, smartphones and the latest computers can display the electronic Instructions for Use directly in PDF format.

On older computers you will need a software program to display the PDF documents such as Adobe Acrobat Reader. You can obtain Adobe Acrobat Reader from the Adobe website.

Spindiag website: ifu.spindiag.de
Adobe website: www.adobe.com

1.3 Manufacturer information

Spindiag GmbH
Engesserstr. 4a
79108 Freiburg, Germany
Tel: +49 761 600 49 600
url: www.spindiag.de

If you need support on technical issues or training topics, please contact us by e-mail at support@spindiag.de or by telephoning +49 761 600 49 600.

For orders and other sales inquiries please contact us by e-mail at orders@spindiag.de or by telephoning +49 761 600 49 600.

- Please have the serial number of your Rhonda player to hand when contacting us. This is located on the nameplate (see Spindiag Rhonda player – Instructions for Use Fig. 3.2 14).
- Additional sets of 20 Rhonda SARS-CoV-2 disks can be ordered under catalog number SD003-02-020-A01-EN.

1.4 Product names

These *Instructions for Use* refer to the following products.

Rhonda player

Processing device

Rhonda app

Optional app for tablets and the Rhonda player

Rhonda disk

Generic product name for all Rhonda disks

Rhonda SARS-CoV-2 disk

Product name for Rhonda disk for the SARS-CoV-2 test.

For convenience, we use the following short form instead of the full name of the product:
SARS-CoV-2 disk.

Table of Contents

2 Analysis procedure

To perform an analysis with the SARS-CoV-2 disk, the user should insert a suitable swab sample (see chapter 3.2 of the Rhonda SARS-CoV-2 disk – Instructions for Use), after resuspension in the swab kit's transport medium, into a SARS-CoV-2 disk. After resuspension the swab transfers approximately 130 µL of sample liquid when inserted into the SARS-CoV-2 disk. The fully automated analysis procedure then begins in the SARS-CoV-2 disk, starting with thermomechanical cell lysis of the sample volume and followed by reverse-transcription (RT) of the released RNA to cDNA. This cDNA is then preamplified for 15 PCR cycles (polymerase chain reaction) as a first step. This is followed by the SARS-CoV-2-specific detection by real-time PCR in three separate main amplifications, i.e. in three sub-volumes as "technical replicates".

3 Internal controls

The SARS-CoV-2 disk uses an integrated MS2 bacteriophage as an internal control ("internal positive control", IPC). The MS2 bacteriophage is a single-stranded RNA virus precharged in dry format in the SARS-CoV-2 disk. The internal control is used to ensure that all the analysis procedures are completed properly, specifically that all components of the RT-PCR are working properly and the required analysis conditions (temperatures and times) are within the validated ranges and the microfluidic processing on the SARS-CoV-2 disk runs within the validated ranges. This control also detects potential RT-PCR inhibitors introduced by the sample.

The control passes through the entire process as follows: Reverse transcription, PCR preamplification followed by real-time PCR main amplification. At the main amplification stage the control undergoes parallel coamplification with each SARS-CoV-2-specific reaction and the control's fluorescence signal is measured in a separate fluorescence channel.

A sensor embedded in the Rhonda player also ensures that the temperatures and holding times required for lysis are achieved and kept within the validated ranges.

The IPC is valid if it meets the validated acceptance criteria.

4 Interpretation of results

Analysis of real-time PCR signals by the Rhonda player is fully automated. If the real-time PCR fluorescence signal exceeds a threshold during a cycle, a Ct value (cycle threshold) is generated. The Ct value is rated as positive/valid if it is within the valid range. Test results are calculated as follows:

Positive

- At least one of the three SARS-CoV-2-specific real-time PCR reactions were rated positive.
- IPC signals are ignored since at least one SARS-CoV-2-specific real-time PCR reaction was rated positive.

Negative

- None of the three SARS-CoV-2-specific real-time PCR reactions was rated positive.
- At least one IPC signal must have been rated as valid.

Invalid

- None of the three SARS-CoV-2-specific real-time PCR reactions was rated positive.
- No IPC signal was rated as valid.

See also the relevant chapters on the test results in the Rhonda SARS-CoV-2 disk Instructions for Use and the Rhonda player Instructions for Use.

5 Analytical performance characteristics

5.1 Analytical sensitivity (limit of detection)

The limit of detection (LoD) is the lowest concentration at which a positive sample can be distinguished from a negative sample with 95% certainty. The limit of detection for the SARS-CoV-2 disk was determined by spiking an artificial matrix (mucin, *Staphylococcus epidermidis*, EDTA whole blood, NaCl, artificial saliva, urea, RNAseA in Amies medium) with decreasing concentrations of SARS-CoV-2 reference material (AccuPlex™ SARS-CoV-2, SeraCare) and analyzing the spiked sample with three different lots of SARS-CoV-2 disks with 24 replicates per concentration each. The limit of detection for the SARS-CoV-2 disk was determined with 7047 copies/mL by probit regression analysis (equivalent to approx. 1007 copies per swab sample).

5.2 Analytical specificity (exclusivity)

Analytical specificity (exclusivity) was determined by testing viruses and microorganisms (see **Tab. 5.1**, **Tab. 5.2**) that are common pathogens for respiratory tract infections or pathogens closely related to the SARS Corona Virus-2. Genomic nucleic acid or viruses were used at a minimum concentration of 1×10^5 copies/swab sample (~7.7×10^5 copies/mL). The measurements were performed in Amies medium. Test results were negative for all pathogens tested. There is thus no indication of cross-reactivity.

Tab. 5.1 Analytical specificity – summary of viruses tested

Viruses
Human Coronavirus 229E
Human Coronavirus OC43
Human Coronavirus HKU1
Human Coronavirus NL63
SARS Corona virus
MERS Corona virus
hMPV
Influenza A (H1N1)
Influenza A (H3N2)
Influenza B (Victoria lineage)
Influenza B (Yamagata lineage)**
Enterovirus D68
Enterovirus 71
Parainfluenza virus 1
Parainfluenza virus 2
Parainfluenza virus 3*
Parainfluenza virus 4b*
Respiratory syncytial virus A
Herpes Simplex virus 1
Adenovirus serotype 1 (C1)*
Adenovirus serotype 7*
Rhinovirus A (HRV 16)
Rhinovirus B (HRV 17)

Tab. 5.2 Analytical specificity – summary of microorganisms tested

Microorganisms
Chlamydia pneumoniae
Haemophilus influenzae
Legionella pneumophilia subsp. Pneumophila
*Streptococcus pneumonia**
Mycobacterium tuberculosis
Streptococcus pyogenes
Bordetella pertussis
Mycoplasma pneumoniae

*cross-reactivity for the SARS-CoV-2 test was excluded here based on tests outside the Rhonda system.

**cross-reactivity was excluded here on the basis of *in silico* analyses by individual cross-checking of the primers and probes of the Rhonda SARS-CoV-2 test against the sequences downloaded from the NCBI database.

5.3 Interfering substances

The effect of potential interfering substances on the functionality of the SARS-CoV-2 disk was determined by preparing and analyzing the following samples with potential interfering substances: the potential interfering substances listed in Tab. 5.3 were dissolved in Amies medium and spiked with SARS-CoV-2 reference material (AccuPlex™ SARS-CoV-2, SeraCare). The potential interfering substances were introduced at concentrations that are likely to exceed the concentration of the substance in an authentic oropharyngeal swab sample. The results from the potential interfering substances study did not reveal any reportable interferences with the chemical substances tested with the exception of acetyl salicylic acid (Aspirin® Plus C 500 mg, Bayer Vital GmbH) which showed inhibition in the Rhonda SARS-CoV-2 test at a very high concentration of 416.7 µg/mL.

Tab. 5.3 Potential interfering substances tested

Substance (manufacturer)	Final concentration
NasenSpray-ratiopharm® adults 15 mL (ratiopharm GmbH)	2.0% (v/v)
Rhinomer® nasal spray 20 mL (Laboratoire de la Mer)	2.0% (v/v)
Entecavir "Baraclude® 0.5mg" (Zentiva Pharma GmbH)	0.4 µg/mL
Tenofovir "Viread® 245mg" (ALIUD Pharma GmbH)	204.2 µg/mL
Budenosid "Rhinocort® aqua" (Hexal AG)	2.0% (v/v)
Tobramycin "TobraZid®" (INFECTOPHARM Arzneimittel und Consilium GmbH)	4.0 µg/mL
Nasacort® 55 µg/dose (Sanofi)	2% (v/v)
Nasonex® (MSD Sharp & Dohme GmbH)	2% (v/v)
Listerine® Total Care 600 mL (Johnson & Johnson)	2% (v/v)
Pantoprazol "Gastrozol® 40mg" (1A Pharma GmbH)	33.4 µg/mL
Acetyl salicylic acid "Aspirin® Plus C 500mg" (Bayer Vital GmbH)	416.7 µg/mL
Ibuprofen "IBU-ratiopharm® 400 mg" (ratiopharm GmbH)	333.4 µg/mL
Paracethamol-ratiopharm® 500 mg (ratiopharm)	416.7 µg/mL
Aciclovir "Aciclovir-ratiopharm® 200 mg" (ratiopharm GmbH)	166.7 µg/mL
Relenza 5 mg	4.2 µg/mL

5.4 Carry-over

To investigate potential carry-over or cross-contamination between positive and negative samples, highly positive (>1 × 10^7 copies/mL) and negative sample were processed in parallel or consecutively in a Rhonda player. The highly positive samples were produced by spiking Amies medium with Twist Synthetic SARS-CoV-2 RNA control (Twist Bioscience), with pure Amies medium used for the negative samples. The scope of measurement comprised 144 samples (36 highly positive samples, 36 negative samples for parallel processing; 36 highly positive samples, 36 negative samples for consecutive processing). The study investigating carry-over and cross-contamination between positive and negative samples did not reveal any carry-over or cross-contamination in Rhonda SARS-CoV-2 disks.

5.5 Repeatability

Repeatability of the Rhonda SARS-CoV-2 test was tested by conducting measurements under constant measurement conditions (one user, one lot of Rhonda SARS-CoV-2 disks, one Spindiag Rhonda player, on one day). The repeatability of the SARS-CoV-2 disk was determined by spiking an artificial matrix (mucin, *Staphylococcus epidermidis*, EDTA whole blood, NaCl, artificial saliva, urea, RNAseA in Amies medium) with various concentrations of SARS-CoV-2 reference material (AccuPlex™ SARS-CoV-2, SeraCare) (spiked samples): negative, weak positive (1.5 × LoD) and moderate positive (3 × LoD). The repeatability study did not reveal any anomalies and the results are summarized in **Tab. 5.4**.

Tab. 5.4 Repeatability study

Sample	Number of samples	Number positive	Percent positive
negative	20	0	0%
~ 1.5 times LoD	20	20	100%
~ 3 times LoD	19	19	100%

5.6 Reproducibility

Reproducibility of the Rhonda SARS-CoV-2 test was tested by conducting measurements under varied measurement conditions: three different users (operators), three different lots of Rhonda SARS-CoV-2 disks, and three different sites. An artificial matrix (mucin, *Staphylococcus epidermidis*, EDTA whole blood, NaCl, artificial saliva, urea, RNAseA in Amies medium) was spiked with the following concentrations of SARS-CoV-2 reference material (AccuPlex™ SARS-CoV-2, SeraCare) (spiked samples): negative, weak positive (1.5 × LoD) and moderate positive (3 × LoD) and used for the measurements. All measurements matched the expected result (100% match) and, taken in conjunction with the measurements for repeatability, confirmed that the SARS-CoV-2 disk in the Rhonda player provides highly reproducible test results when the same samples are tested in multiple runs, by different users, at different sites, and using different lots of Rhonda SARS-CoV-2 disks. The results of the reproducibility study are summarized in Tab. 5.5 through Tab. 5.7.

Tab. 5.5 Study of reproducibility (operator)

Operator	Sample	Number of samples	Number positive	Percent positive
Operator 1	negative	18	0	0%
	~ 1.5 times LoD	18	18	100%
	~ 3 times LoD	18	18	100%
Operator 2	negative	18	0	0%
	~ 1.5 times LoD	18	18	100%
	~ 3 times LoD	18	18	100%
Operator 3	negative	18	0	0%
	~ 1.5 times LoD	18	18	100%
	~ 3 times LoD	18	18	100%

Tab. 5.6 Study of reproducibility (lot)

Operator	Sample	Number of samples	Number positive	Percent positive
Lot 1	negative	18	0	0%
	~ 1.5 times LoD	18	18	100%
	~ 3 times LoD	18	18	100%
Lot 2	negative	18	0	0%
	~ 1.5 times LoD	18	18	100%
	~ 3 times LoD	18	18	100%
Lot 3	negative	18	0	0%
	~ 1.5 times LoD	18	18	100%
	~ 3 times LoD	18	18	100%

Tab. 5.7 Study of reproducibility (site)

Operator	Sample	Number of samples	Number positive	Percent positive
Site 1	negative	18	0	0%
	~ 1.5 times LoD	18	18	100%
	~ 3 times LoD	18	18	100%
Site 2	negative	18	0	0%
	~ 1.5 times LoD	18	18	100%
	~ 3 times LoD	18	18	100%
Site 3	negative	18	0	0%
	~ 1.5 times LoD	18	18	100%
	~ 3 times LoD	18	18	100%

6 Clinical performance characteristics

The clinical performance characteristics of the Rhonda SARS-CoV-2 disk were determined by analyzing oropharyngeal swab samples (clinically negative samples, clinically positive samples and spiked clinical samples). Spiked samples were spiked with SARS-CoV-2 reference material (AccuPlex™ SARS-CoV-2, SeraCare). The samples were analyzed in parallel with a comparator system (cobas® 6800 or cobas® 8800, Roche Diagnostics International AG) and the test results were then compared. The results obtained from the Rhonda SARS-CoV-2 disk are shown in Tab. 6.1.

Tab. 6.1 Comparison of Rhonda SARS-CoV-2 disk and comparator system regarding sample identification

Sample type	Rhonda SARS-CoV-2 disk (detected as positive/total)	Comparator system (detected as positive/total)
Spiked samples	33/35**	32/35*
Clinically positive samples	4/4	4/4
Clinically negative samples	0/51	0/51

*Three samples at the LoD were not detected as positive by the comparator system.

**One sample at the LoD and an additional sample were not detected as positive by the Rhonda SARS-CoV-2 disk.

The performance parameters for the Rhonda SARS-CoV-2 disk were determined from the study of clinical performance characteristics, based on sample identification (positive/negative) (Tab. 6.2) with spiked samples and clinically positive samples assumed to be positive and clinically negative samples assumed to be negative. The results were also analyzed using the comparator system as reference (Tab. 6.3) with samples that tested positive with the comparator system assumed to be positive and samples that tested negative with the comparator system assumed to be negative.

Clinical sensitivity was calculated as 100% × (TP/(TP+FN)), clinical specificity as 100% × (TN/(TN+FP)), PPV (positive predictive value) as TP/(TP+FP) and NVP (negative predictive value) as TN/(TN+FN), where TP = true positive, FP = false positive, TN =true negative and FN = false negative.

Tab. 6.2 Clinical parameters based on sample identification

Parameter	Value	95% confidence interval
Clinical sensitivity	94.87%	83.12 – 98.59%
Clinical specificity	100.00%	93.00 – 100%
PPV	100.00%	not available
NPV	96.30%	not available

Tab. 6.3 Clinical parameters with comparator system as reference

Parameter	Value	95% confidence interval
Clinical sensitivity	97.22%	85.83 – 99.51%
Clinical specificity	96.30%	87.47 – 98.98%
PPV	94.59%	not available
NPV	98.11%	not available

7 Trademarks

AccuPlex™ SARS-CoV-2 (SeraCare Life Sciences, Inc.), NasenSpray-ratiopharm® adults (ratiopharm GmbH), Rhinomer® nasal spray (Laboratoire de la Mer), Entecavir "Baraclude® 0,5mg" (Zentiva Pharma GmbH), Tenofovir "Viread® 245mg" (ALIUD Pharma GmbH), Budenosid "Rhinocort® aqua" (Hexal AG), Tobramycin "TobraZid®" (INFECTOPHARM Arzneimittel und Consilium GmbH), Nasacort® 55 µg/dose (Sanofi), Nasonex® (MSD Sharp & Dohme GmbH), Listerine® Total Care (Johnson & Johnson), Pantoprazol "Gastrozol® 40mg" (1A Pharma GmbH), Acetyl salicylic acid "Aspirin® Plus C 500mg" (Bayer Vital GmbH), Ibuprofen "IBU-ratiopharm® 400 mg" (ratiopharm GmbH), Paracethamol-ratiopharm® 500 mg (ratiopharm), Aciclovir "Aciclovir-ratiopharm® 200 mg" (ratipharm GmbH), Twist Synthetic SARS-CoV-2 RNA control (Twist Bioscience), cobas® 6800 and cobas® 8800 (Roche Diagnostics International AG). Registered names, trademarks, etc., used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

For your notes ...

For your notes ...


carbon neutral
natureOffice.com | DE-616-JRPJL66
print production