



# **Approach to sensitivity evaluation of SARS-CoV-2 antigen rapid diagnostic tests (Ag RDT)**

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# COVID-19 IVDs



## Regulation of IVDs in Europe: in transition...



### IVD Directive (2000 – May 2022)

- SARS-CoV-2 IVD = Annex III products: „low risk“
- Self-Certification of SARS-CoV-2 IVD by manufacturer, basic requirements
  - (exception: self-tests; Notified Body assessment of lay man study)



### IVD Regulation (from May 2022)

- SARS-CoV-2 IVD = Class D, highest risk group
- Certification by Notified Body + EU Reference Laboratory
- Distribution of COVID-19 IVD under old rules possible until May 2025



# SARS-CoV-2 Ag RDT



## Approach in Germany

**MoH:** reimbursement of SARS-CoV-2 Ag RDT by German health care system linked to „**minimal quality criteria**“ (manufacturer)

- **Sensitivity:** >80%
  - >100 unselected PCR pos, <7 days after symptoms
- **Specificity:** >97%
  - >100 asymptomatic individuals w/o exposure risk
- **Potential cross reactivity**

combined with ....

**Comparative sensitivity evaluation (PEI / RKI)**

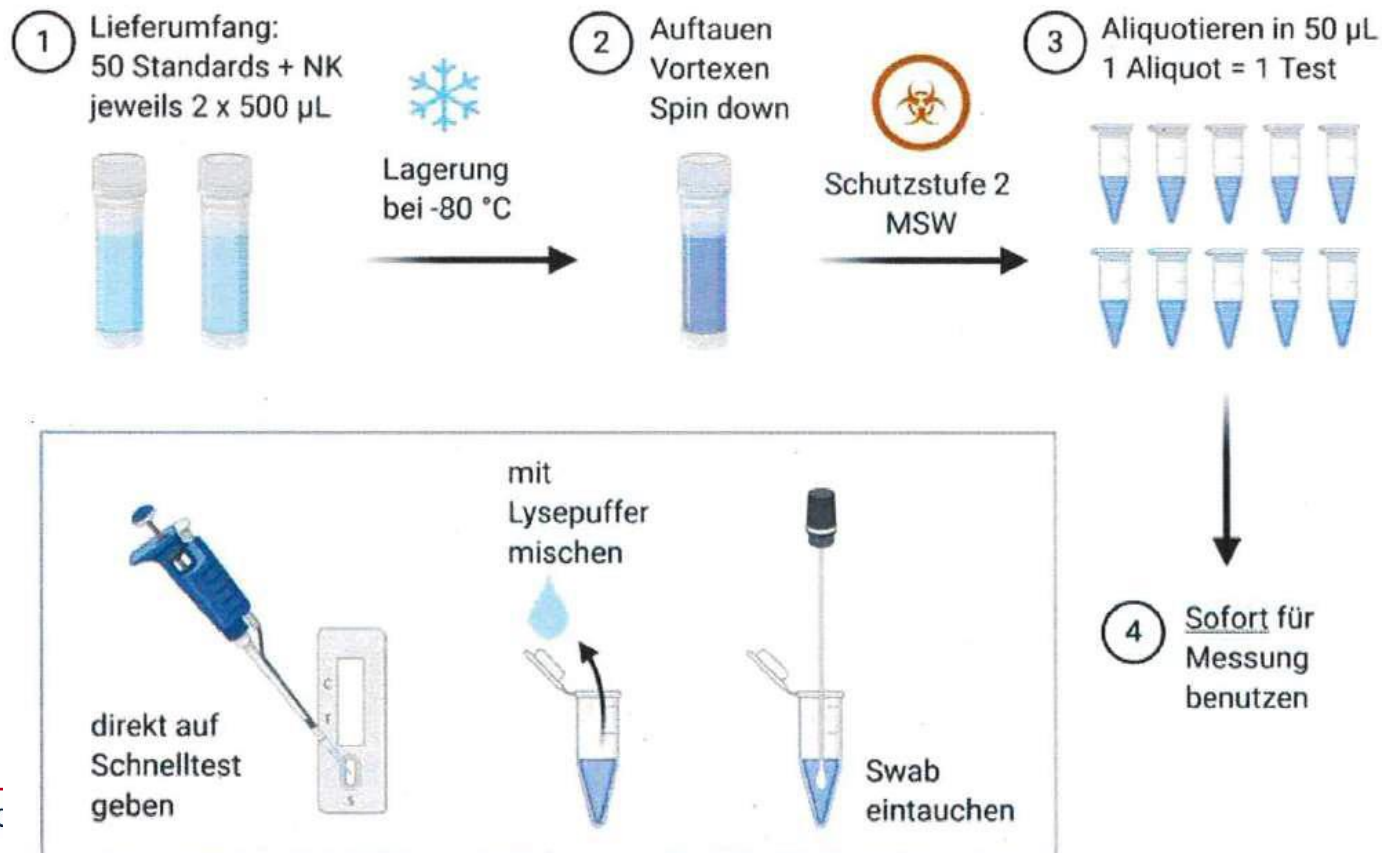
## Standardized multi-center comparative evaluation (PEI, RKI)

- **Evaluation Panel** (n=50 members)
  - 50 pools, eluted from <500 pharyngeal swabs (PBS)
  - Some pools with small amount of VTM
  - Viral load range determined by PCR
    - CT 17 – 36                      6 orders of magnitude
    - CT 25  $\approx$  1 mio RNA copies / mL)
  - Cell culture: infectious virus still detectable from samples with CT<25
  - 500  $\mu$ l pools kept frozen at -80°C
  - After thawing, use of 50  $\mu$ l aliquots within max. 5 days
  - SARS-CoV-2 antigen stable at +4°C for one week

# Test Procedure

## ■ Testing

- Inclusion of pre-analytical steps
  - Incubation of test-specific swabs in 50µl pool aliquots
  - Elution as described in the IFU



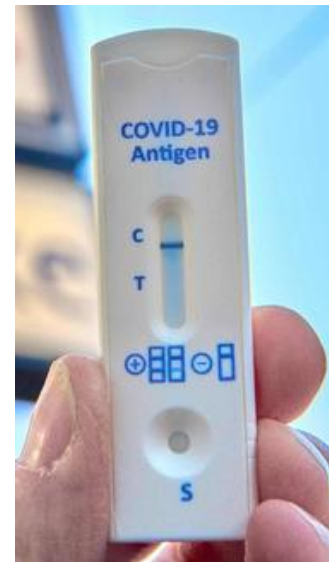
- **Test interpretation**
  - Independant visual reading by two persons
    - discrepant results (=reactive / non-reactive) in favor of test interpreted as „pos“
  - Test validity; control line reactive
  - Documentation by blot reader and foto
  
- **Criteria**
  - >75% detection rate for panel members with high viral loads (CT<25)

# SARS-CoV-2 Ag RDT



## Comparative Sensitivity Evaluation (since Nov 2020)

- 226 tests evaluated (October 2021)
  - Target antigen: 95,7% N                      2,5% S                      1,8% N + S
- >75% detection rate for CT<25
  - **183 pass**                      81%
  - **43 fail**                      19%



# SARS-CoV-2 Ag RDT: State of Art



## 226 tests evaluated (October 2021)

### 183 pass

Ag content

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Manufacturer		Abbott Rapid Diagnostic Jena GmbH	ACON Witech (Hangzhou) Co., Ltd	Aekis Diagnostics GmbH	Affimedix	Amazing Witech (Shanghai) Co., Ltd	Amade Labor Diagnostik GmbH	AmorMed (Klausen) Biotechnology Co., Ltd.	Aubio (Klausen) Biotechnology Co., Ltd.	Anhui Deepblue Medical Technology Co., Ltd.	ASIAN PHARM.CO.,LTD.	ASiC Link Technology Co.,Ltd.	Auslin	AXOM Gesellschaft für Diagnostica und Biochemie mbH	Azure Biotech Inc.	Becton Dickinson	Beijing Biter Biowing Biotech Co., Ltd.	Beijing Hogen Witech Co., Ltd.
Test name		Parbio™ Covid-19 Ag Rapid Test Device	Rowflex SARS-CoV-2-Antigenschnelltest (Nasopharyngeal)	Aekis Rapid SARS-CoV-2 Rapid Test	TectiON® - COVID-19 Antigen	CoroVite Covid-19 Ag Verflüchtigungsstreifen Teststreifen (Kolloidales Gold)	AMP Rapid Test SARS-CoV-2 Ag	COVID-19 Antigen Rapid Test Kit (Kolloidales Gold)	Rapid Covid-19 Antigen Test (Kolloidales Gold)	COVID-19 (SARS-CoV-2) Antigen Test Kit (Kolloidales Gold)	Asian Easy Test COVID-19 Ag	Nova Test SARS-CoV-2 Antigen Rapid Test Kit	Komart® SARS-CoV-2 Antigen Rapid Test	Axiom Diagnostica COVID-19 Ag Schnelltest	Ona Sure Covid-19 Antigen Rapid Test Device (Nasopharyngeal/Oral)	BD Veritor™ System for Rapid Detection of SARS-CoV-2	Covid-19 Antigen Schnelltest	NeuTigen Coronavirus (2019-nCoV)-Antigen-Test (Kolloidales Gold)
Panel 1 V1		Panel 1 V2																
Panel No.	CT	Panel No.	CT	Panel 1 V1	Panel 1 V2	Panel 1 V1	Panel 1 V2	Panel 1 V1	Panel 1 V2	Panel 1 V1	Panel 1 V2	Panel 1 V1	Panel 1 V2	Panel 1 V1	Panel 1 V2	Panel 1 V1	Panel 1 V2	Panel 1 V2
1	17,55	1	17,61															
6	20,20	2	19,08															
5	20,28	3	19,62															
8	20,88	5	20,80															
2	20,94	4	20,60															
4	20,98	6	21,21															
7	21,71	12	22,12															
12	21,82	7	22,35															
8	21,95	8	22,12															
9	22,14	16	22,88															
11	22,96	9	23,18															
16	22,55	11	23,18															
10	22,88	10	23,21															
14	23,42	15	24,48															
17	24,00	23	24,45															
23	24,04	17	24,61															
15	24,16	20	24,61															
18	24,28	25	25,11															
27	25,14	24	25,20															
26	25,24	18	25,29															
41	25,27	19	25,45															
19	25,40	21	25,45															
26	25,67	27	26,12															
19	25,90	31	26,26															
21	25,94	26	26,32															
22	25,87	32	26,64															
30	26,27	35	26,66															
32	26,44	36	27,05															
35	27,04	30	27,38															
28	27,14	29	27,46															
29	27,15	28	27,41															
40	27,45	22	27,62															
36	27,89	34	27,82															
36	28,14	40	28,19															
38	28,14	18	28,33															
42	28,63	33	28,92															
30	28,96	38	29,26															
33	28,96	20	29,45															
44	29,34	42	29,68															
35	29,70	44	29,51															
39	29,76	39	30,12															
45	30,10	37	30,18															
41	30,18	41	30,18															
37	30,54	45	31,19															
43	31,05	48	31,19															
46	31,54	46	31,46															
48	32,06	43	31,61															
47	35,19	47	34,55															
49	35,22	50	35,33															
50	36,46	49	36,04															

>75%



# SARS-CoV-2 Ag RDT: State of Art



## 226 tests evaluated (October 2021)

### 43 fail

Ag content

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Manufacturer		Acro Biotech Inc	Aikang Diagnostics Co., Ltd.	Beijing Wantai Biotechnology Co., Ltd.	Ceritex/ Biotec S. L.	Coris Bioconcept	Hangzhou Alltest Biotech Co., Ltd.	Hangzhou Biotest Biotech Co., Ltd.	Hangzhou Genewell Biotech Co., Ltd.	Hangzhou Realy Tech Co., Ltd.	Israk International Trading	Jinsheng Biomedical Technology Co., Ltd.	Joybio (Tianjin) Biotechnology Co., Ltd.	Lonex GmbH	Medicon Co., Ltd.	Microware GmbH Heidelberg	nal von minden GmbH	Rapigen
Test name		Acro COVID-19 Antigen Rapid Test	SARS-CoV-2 Antigen Test Kit (Immunochromatography)	New Coronavirus (SARS-CoV-2) N Protein Detection Kit (Fluorescence Immunochromatography)	Ceritex Biotec SARS-CoV-2 Ag Test	COVID-19 Ag Rapid Strip	COVID-19 Ag Alltest	Luminatek SARS-CoV-2 Antigen Rapid Test Cassette	ROKUI COVID-19 Antigen Rapid Test Device	Novel Coronavirus (SARS-CoV-2) Antigen Rapid Test Cassette (swab)	Bioek medical COVID-19 Antigen Rapid Test Cassette	COVID-19 Antigen Rapid Test (swab)	Joybio SARS-CoV-2 Antigen Rapid Test Kit (Colloidal Gold)	Lonex COVID-19 Ag Rapid Test	TrueLine COVID-19 Ag Rapid Test	QuickDetection COVID-19 Antigen Schnelltest	dedico Medical Test COVID-19 Ag plus Test	Bioecdt COVID-19 Ag
Panel 1 V1	Panel 1 V2	Panel 1 V1	Panel 1 V2	Panel 1 V1	Panel 1 V2	Panel 1 V1	Panel 1 V1	Panel 1 V2	Panel 1 V2	Panel 1 V2	Panel 1 V2	Panel 1 V2	Panel 1 V2	Panel 1 V2	Panel 1 V2	Panel 1 V2	Panel 1 V2	Panel 1 V1
1	17,35	1	17,35	1	17,35	1	1	1	1	1	1	1	background	1	background	1	1	1
6	20,20	2	19,28	0	1	0	1	1	0	1	1	1	background	1	background	1	1	1
5	20,28	3	19,62	0	0	0	0	1	1	1	1	1	background	1	background	1	1	1
3	20,28	5	20,28	0	0	0	1	1	1	1	1	1	background	1	background	1	1	1
2	20,54	4	20,61	0	0	0	1	1	1	1	1	1	background	1	background	1	1	1
4	20,68	6	21,21	0	0	0	1	1	1	1	1	1	background	1	background	1	1	1
7	21,71	12	22,12	0	0	0	0	0	0	1	1	0	background	1	background	0	0	0
12	21,82	7	22,35	0	0	0	0	0	0	1	1	1	background	0	background	1	1	0
8	21,95	8	22,62	0	0	0	0	0	0	1	0	1	background	1	background	0	0	0
9	22,54	16	22,88	1	0	0	0	0	1	0	0	0	background	0	background	1	0	1
11	22,94	9	23,18	0	0	0	0	0	0	1	1	1	background	0	background	1	1	0
10	23,11	11	23,18	0	0	0	0	0	0	0	0	0	background	0	background	1	0	0
13	23,11	10	23,21	1	0	0	0	0	0	1	0	0	background	0	background	0	0	0
14	23,11	15	24,08	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
17	24,00	23	24,05	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
23	24,04	17	24,21	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
15	24,14	14	24,27	0	0	0	0	0	0	0	0	0	background	0	background	0	1	0
16	24,14	25	24,27	0	0	0	0	0	0	0	0	0	background	0	background	1	0	0
27	25,24	24	25,25	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
24	25,24	18	25,29	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
32	25,27	19	25,45	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
18	25,80	21	25,85	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
26	25,87	27	26,12	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
19	25,90	31	26,34	0	0	0	0	0	0	0	0	0	background	1	background	0	0	0
21	25,94	26	26,32	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
22	25,87	32	26,84	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
20	26,27	35	26,86	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
32	26,84	36	27,05	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
35	27,04	30	27,28	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
28	27,14	29	27,34	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
29	27,15	28	27,41	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
40	27,40	22	27,42	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
34	27,89	34	27,82	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
36	28,13	40	28,19	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
38	28,14	13	28,33	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
42	28,63	33	28,92	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
30	28,86	38	29,36	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
33	28,96	20	29,66	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
44	29,24	42	29,68	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
25	29,70	64	29,11	0	0	0	0	0	0	0	0	0	background	0	background	0	1	0
39	29,76	39	30,12	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
45	30,10	37	30,13	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
41	30,13	41	30,13	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
37	30,54	45	31,19	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
43	31,05	48	31,19	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
46	31,54	46	31,34	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
48	32,06	43	31,61	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
47	35,19	47	34,55	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
49	35,22	50	35,33	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0
50	36,36	49	36,04	0	0	0	0	0	0	0	0	0	background	0	background	0	0	0

>75%



# Publications

## (1) Design and manufacture panel

***Puyskens A, Krause E, Michel J, Nübling CM, Scheiblaue H, Bourquain D, Grossegese M, Valusenko R, Corman VM, Drosten C, Zwirgmaier K, Wölfel R, Lange C, Kramer J, Friesen J, Ignatius R, Müller M, Schmidt-Chanasit J, Emmerich P, Schaade L, Nitsche A.***

Establishment of a specimen panel for the decentralised technical evaluation of the sensitivity of 31 rapid diagnostic tests for SARS-CoV-2 antigen, Germany, September 2020 to April 2021. Eurosurveillance 2021 (accepted)

## (2) Comparative evaluation

***Scheiblaue H, Filomena A, Nitsche A, Puyskens A, Corman VM, Drosten C, Zwirgmaier K, Lange C, Emmerich P, Müller M, Knauer O, Nübling CM.***

Comparative sensitivity evaluation for 122 CE-marked rapid diagnostic tests for SARS-CoV-2 antigen, Germany, September 2020 to April 2021. Eurosurveillance 2021 (accepted)

**Comparative Sensitivity Evaluation of 226 products** (Nov 2020-Oct 2021)  
using standardized panel of 50 members (CT 17 – CT 36)

- SARS-CoV-2 RDT with broad continuous sensitivity range (0/50 – 43/50 pos)
- „state of the art“ *„75% for CT<25“*
- Pre-analytical steps critical
- Batch-to-batch consistency
- Same original products often provided under different names, traceability
- Comparative evaluation continued (panel version 3)

**Comparative Sensitivity Evaluation of 226 products** (Nov 2020-Oct 2021)  
using standardized panel of 50 members (CT 17 – CT 36)

- Potential bias, e.g. PBS as diluent ?
  - Results of all assays follow analyte concentration
  - Pools directly in elution buffer (w/o swab) results in higher sensitivity (Puyskens et al)
  
- Biological assays best standardized by biological material
  - native SARS-CoV-2 from respiratory specimens
  
  - Cell culture supernatant: clumping (?)



# Thank you for your attention !!

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