LABORATORY · DIAGNOSTICS

ANA plus ViraChip® IgG Test Kit

Instructions for Use

CE

Multiplex autoantibody assay in microarray format for the qualitative detection of **IgG** autoantibodies against specific **nuclear** and **cytoplasmic** antigens in human serum as part of autoimmune diagnostics.

The **ANA plus ViraChip® IgG Test Kit** is a microarray based on an enzyme-linked immunosorbent assay (ELISA) that utilizes the following purified, specific antigens: **dsDNA, nucleosome, PCNA, P0, SS-A 52, SS-A 60** (Ro)**, histone, Sm, Sm/RNP, Ku, SS-B** (La)**, CENP B, Scl-70, PM/Scl, Jo-1, Mi-2**, and **DFS70**. Additionally, a **DNA specific control** is included to evaluate the specific reactivity of the dsDNA spot duplet.

The ANA plus ViraChip® IgG Test Kit is manufactured in accordance with Directive **98/79/EC**.

Principle of the assay

At the bottom of each well of a standard 96-well microtiter plate (MTP) is a nitrocellulose membrane on which antigens are immobilized as analyte spots (microarray). During serum incubation, nuclear and cytoplasmic autoantibodies in the patient's serum bind to the immobilized antigens on the microarray. During the conjugate incubation, the conjugate binds to the antigen-antibody complex. After adding the Chromogen/Substrate solution, the alkaline phosphatase bound to the conjugate-antibody complex converts the Chromogen/Substrate, thereby staining the antigen-antibody complex on the microarray purple to black. After the serum incubation, the conjugate incubation, and the Chromogen/substrate incubation, a washing step is performed in each case to remove unbound antibodies and reagents.

In addition to the analyte spots, each microarray contains the following control spots: **serum controls**, **conjugate controls, calibrator controls**, and a **negative control**. The analyte spots are used to detect antibodies against the nuclear and cytoplasmic antigens **dsDNA, nucleosome, PCNA, P0, SS-A 52, SS-A 60** (Ro)**, histones, Sm, Sm/RNP, Ku, SS-B** (La)**, CENP B, Scl-70, PM/Scl, Jo-1, Mi-2**, and **DFS70.** In addition, a **DNA specific control** is applied to evaluate the specific reactivity of the dsDNA spot duplet.

For clear identification, the wells are color-coded: The ANA plus ViraChip® IgG is marked with a **solid orange circle** on the edge of the well.

Order No.:	**V-APCGOK**	Order No.:	**V-APCGDK (Deca Kit)**
Kit size:	**1 MTP with 96 single breakable wells**	Kit size:	**10x 1 MTP with 96 single breakable wells**
Specimen:	**10 µl serum**	Specimen:	**10 µl serum**
Time for testing:	**approx. 130 minutes**	Time for testing:	**approx. 130 minutes**

Materials provided

1x or 10 MTPs, each with 96 wells	**ANA plus ViraChip® IgG Antigen Coated Wells** Single breakable wells with ViraChip® microarrays, ready to use	(Prod. No.: V-APCGAC)
1x or 10x 12 ml	**ViraChip® AP-Anti-Human IgG Conjugate** Anti-human IgG conjugate solution for ViraChip® tests, derived from goat, with bovine alkaline phosphatase, ready-to-use	(Order No.: V-UVCGKI)
1x or 10x 100 mL	**ViraChip® / ViraStripe® / ViraBlot® Diluent / Wash Buffer** Wash Buffer Concentrate for ViraChip® tests, containing detergents and salts to prevent nonspecific binding, 10x	(Order No.: V-UVNUWP)
1x or 10x 12 mL	**ViraChip® Chromogen / Substrate Solution** Chromogen/Substrate Solution for ViraChip® tests, containing BCIP/NBT, ready-to-use	(Order No.: V-UVCUCS)
1x or 10x 50 ml	**ViraChip® Sample Buffer** Sample buffer for ViraChip® tests, containing milk powder, detergents, and salts to prevent nonspecific binding, ready-to-use	(Order No.: V-UVCUPP)

Optionally available

1 MTP strip with 8 wells	**ANA plus ViraChip® IgG Antigen Coated Wells (8)** Single breakable wells with ViraChip® microarrays, ready to use	(Order No.: V-APCGRT)
330 µl	**ANA plus ViraChip® IgG Positive Control** IgG positive control serum, human, ready-to-use	(Order No.: V-ANCGPK)
330 µl	**ANA plus ViraChip® IgG Negative Control** IgG-negative control serum, human, ready-to-use	(Order No.: V-ANCGNK)

Additional equipment required

1. **ViraChip® Software®**	for preparing, performing, and evaluating the test run, version 1.3.0-1960 or later	(Order No.: V-VCNUPR)
2. **2D barcode scanner**	for reading the DataMatrix codes	
3. **Microtiter plate** with 96 empty wells	for refilling partially used strips of the microtiter plate in the holding frame	(Order No.: V-UVNMTP)
4. **Orbital shaker (750 rpm)** or **linear shaker (20 Hz)**	for mixing samples and reagents during processing	
5. **ViraChip® Scanner** or **ViraChip® Reader**	for digitizing/measuring developed ViraChip® tests, starting with ViraChip® Scanner v1.0 or ViraChip® Reader Rev.01	(Order No.: V-UVCSCA) (Order No.: V-UVCCAM)
6. Optional: **Fan**	for faster drying of developed microtiter plates	

Preparation of Reagents and Patient Samples

Bring all reagents and the packaged microtiter plate to room temperature (RT: 20–23°C) before use. Mix all reagents thoroughly before use.

Wash buffer solution: Dilute **the wash buffer concentrate 1:10** in distilled or deionized water (H_2O), e.g., 100 mL **working** Wash buffer concentrate + 900 mL H_2O

Sample buffer: Ready to use

Conjugate solution: Ready to use

Chromogen / Substrate Solution: Ready to use

MTP wells: Carefully remove the microtiter plate (MTP) from the packaging and insert the required number of wells into an empty microtiter plate rack (Preparing and Performing the Test Run, Step 3). Use the wells immediately

© Copyright VIRAMED Biotech AG January 2024 0122/0950

VIRAMED BIOTECH AG • Behringstraße 11 • 82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
viramed@viramed.de • www.viramed.de

after removing them from the packaging. Return any unused wells to the original packaging immediately, seal tightly, and store at 2–8°C.

Patient samples: Patient samples are added at a **1:76** dilution, e.g., **10 µl patient sample** + **750 µl sample buffer**. During processing, 100 µl of the diluted sample is required.

Controls: If necessary, the positive and negative control sera are diluted **1:16**, e.g., **10 µl control serum** + **150 µl sample buffer**. During processing, 100 µl of the diluted control is used.

Design of the ANA plus ViraChip® IgG Microarray

Antigens:
The following antigens are applied twice in identical concentrations as spot duplets: **dsDNA, nucleosome, PCNA, P0, SS-A 52, SS-A 60** (Ro)**, histone, Sm, Sm/RNP, Ku, SS-B** (La)**, CENP B, Scl-70, PM/Scl, Jo-1, Mi-2.** In addition, the **DFS70** antigen is applied as a single spot.

Controls:

Each ViraChip® well contains the following integrated controls:
Two serum controls (sc), one negative control (nc), two IgG conjugate controls (ccG), and six calibrator controls (cal). Additionally, a **DNA specific control** is included to evaluate the specific reactivity of the dsDNA spot duplet.

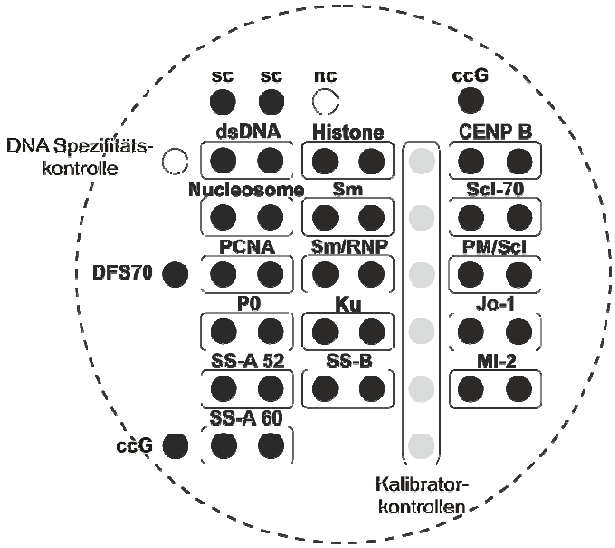


Figure 1: Image of a well from the microtiter plate containing the ANA plus ViraChip® IgG Microarray (enlarged). Arrangement of the spot duplets for antigens and the spots for integrated controls.

Nomenclature of Nuclear and Cytoplasmic Antigens

Antigen:	Remarks:
dsDNA	Double-stranded DNA
Nucleosome	Protein complex
PCNA	Proliferating Cell Nuclear Antigen
P0	Ribosomal phosphoprotein
SS-A 52	Sjögren's Syndrome Antigen A, 52 kDa
SS-A 60	Sjögren's Syndrome Antigen A, 60 kDa
Histone	basic protein
Sm	Smith Antigen
Sm/RNP	Smith antigen/ribonucleoprotein
Ku	DNA-binding, nuclear heterodimer (p70/p80)
SS-B	Sjögren's Syndrome Antigen B
CENP B	Centromere B Antigen
Scl-70	Topoisomerase I
PM/Scl	Exoribonuclease
Jo-1	Histidyl-tRNA synthetase
Mi-2	Nuclear helicase, 235–240 kDa
DFS70	Dense Fine Speckled 70 Antigen, 70 kDa

© Copyright VIRAMED Biotech AG January 2024 0122/0950

VIRAMED BIOTECH AG • Behringstraße 11 • 82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
viramed@viramed.de • www.viramed.de

Preparing and Performing the Test Run

In automated processing, incubation times, volumes, and the order of the individual steps may differ from the procedure described below. Detailed step-by-step instructions for the automation solution used, as well as instructions for using the ViraChip® Software, will be provided by Viramed Biotech AG during equipment setup and training. See also the section “Notes on Equipment and Software.”

1. **Loading**
   Select the test and enter the sample data into the assignment scheme in the ViraChip® Software.

2. **Loading**
   Scan the 2D barcodes on the Test Kit and microtiter plate labels to transfer the lot numbers and lot specific factors.

3. **Processing**
   Perform all processing steps at room temperature (RT).

   3.1 Preparation
   - Place the number of wells specified in the layout into the holding frame.
   - Fill any empty positions of a strip of the microtiter plate in the holding frame with blank wells. The strips are labeled 1–12 on the microtiter plate.

   Be careful not to let any plastic particles fall into the wells when breaking the strips.

   3.2 Preincubation
   - Add 300 µl of ready-to-use wash buffer to each well.
   - Incubate for 5 minutes at room temperature on the shaker.
   - Aspirate the liquid.

   3.3 Serum Incubation
   - Add 100 µl of diluted patient sample or 100 µl of diluted control serum to the corresponding well according to the assignment scheme.
   - Incubate on the shaker at room temperature (RT) for 30 minutes.
   - Aspirate the liquid.

   3.4 Wash 3 times
   - Add 300 µl of ready-to-use wash buffer to each well.
   - Incubate for 5 minutes at room temperature on the shaker.
   - Aspirate the liquid.

   Adjust the aspiration needles or use pipettes in such a way that the bottom of the wells are not damaged.

   3.5 Conjugate Incubation
   - Add 100 µl of conjugate solution to each well.
   - Incubate for 30 minutes at room temperature on the shaker.
   - Aspirate the liquid.

   The well bottoms must be completely covered with liquid during the incubation steps.

   During the incubation and washing steps, use an orbital shaker adjustable to a frequency of approximately 750 rpm, or a linear shaker adjustable to a frequency of approximately 20 Hz.

   3.6 Wash 3 times
   - Add 300 µl of ready-to-use wash buffer to each well.
   - Incubate for 5 minutes at room temperature on the shaker.
   - Aspirate the liquid.

   3.7 Wash once
   - Add 300 µl of distilled or deionized water to each well.
   - Incubate for 1 minute at room temperature.
   - Aspirate the liquid.

   3.8 Substrate Incubation
   - Add 100 µl of Chromogen/substrate solution to each well.
   - Incubate for 15 minutes at room temperature on the shaker.
   - Stop the reaction: Aspirate the liquid.

   3.9 Wash 3 times
   - Add 300 µl of distilled or deionized water to each well.
   - No intermediate incubation.
   - Aspirate the liquid.

   3.10 Dry the wells
   Allow to dry for 20 minutes at a maximum humidity of 60% under a continuous air flow.

   At higher humidity levels, the drying time may be longer. Alternatively, allow to dry for 12 hours at room temperature, protected from light.

4. **Scanning**
   Measure ViraChip® microarrays using the ViraChip® Scanner or the ViraChip® Reader.

   Measurements of the ViraChip® microarrays must be performed within 24 hours after processing (store the microtiter plate protected from light). Please refer to the device manual for detailed instructions.

© Copyright VIRAMED Biotech AG January 2024 0122/0950

VIRAMED BIOTECH AG • Behringstraße 11 • 82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
viramed@viramed.de • www.viramed.de

5. Analyze

5.1 Verify the assignment of antigen spot duplets and control spots (technically validate)

Based on its position, each spot is automatically assigned by the ViraChip® Software to the corresponding antigen spot duplet or control spot.
The automatic assignment must be visually verified against the pattern shown in Fig. 1. Set assignments that do not match the pattern shown to "invalid" in the "QC" field. The corresponding sample must be retested.

5.2 Validation

The validation is performed automatically by the ViraChip® Software.

A ViraChip® microarray is valid if the following control spots meet the criteria stored in the ViraChip® Software:
- **Serum controls** (sc) above the threshold
- **IgG Conjugate Controls** (ccG) above the threshold
  The IgG conjugate controls must react more strongly than the controls for the other conjugate classes.
- **Calibrator controls** (cal) above the threshold
- **Negative control** (nc) below the threshold

If any of the above criteria are not met, the ViraChip® microarray is classified as "invalid." Invalid ViraChip® microarrays must not be evaluated and must be repeated.

5.3 Evaluation of ViraChip® Microarrays

The ViraChip® Software automatically evaluates patient samples according to the interpretation criteria defined below:

The ViraChip® units for each spot duplet are calculated by the ViraChip® Software relative to the calibrator controls, taking lot specific factors into account.

Interpretation criteria

ViraChip® units of the spot duplets	Result	Assessment
At least one spot duplet ≥ **100 ViraChip® units** from: Nucleosome, PCNA, P0, SS-A 52, SS-A 60, Histone, Sm, Sm/RNP, Ku, SS-B, CENP B, Scl-70, PM/Scl, Jo-1, Mi-2.	**Positive**	**Specific autoantibodies** against the respective antigen detectable: **Nucleosome, PCNA, P0, SS-A 52, SS-A 60, Histone, Sm, Sm/RNP, Ku, SS-B, CENP B, Scl-70, PM/Scl, Jo-1, Mi-2.** Indication of an autoimmune disease associated with the respective detectable nuclear or cytoplasmic autoantibodies.
No spot duplet ≥ **100 ViraChip® units** from: Nucleosome, PCNA, P0, SS-A 52, SS-A 60, Histone, Sm, Sm/RNP, Ku, SS-B, CENP B, Scl-70, PM/Scl, Jo-1, Mi-2.	**Negative**	**No** specific autoantibodies or only trace amounts detectable.
dsDNA spot duplet **≥ 100 ViraChip® units** if dsDNA spot duplet > DNA specific control	**Positive**	**Specific autoantibodies** against **dsDNA** are detectable. Indicative of systemic lupus erythematosus.
dsDNA spot duplet **≥ 50 and < 100 ViraChip® units** If dsDNA spot duplet > DNA specific control	**Equivocal**	**Small amounts** of specific autoantibodies against **dsDNA** are present.
dsDNA spot duplet ≥ 50 ViraChip® units **If dsDNA spot duplet < DNA specific control**	**dsDNA spot duplet Cannot be interpreted**	A reaction with the DNA specific control may indicate nonspecific reactions of the dsDNA spot duplet. The dsDNA spot duplet is not interpretable.
dsDNA spot duplet **< 50 ViraChip® units**	**Negative**	**No** specific autoantibodies against **dsDNA** detectable.
DFS70 Spot **≥ 100 ViraChip® units**	**Positive**	**Specific autoantibodies** against **DFS70 are** detectable.
DFS70 Spot **< 100 ViraChip® units**	**Negative**	**No** or only small amounts of specific autoantibodies against **DFS70** detectable.

Diagnostic significance of nuclear and cytoplasmic autoantibodies

1. dsDNA (double-stranded DNA): Autoantibodies against dsDNA (double-stranded DNA, or native DNA) are of very high diagnostic and prognostic significance and serve as markers of disease activity. They are present in approximately 90% of patients with SLE. The detection of autoantibodies against native or dsDNA is considered one of the eleven criteria of the American College of Rheumatology (ACR criteria from 1982, revised in 1997). Autoantibodies against dsDNA are the most commonly detected

© Copyright VIRAMED Biotech AG January 2024 0122/0950

VIRAMED BIOTECH AG • Behringstraße 11 • 82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
viramed@viramed.de • www.viramed.de

SLE-specific autoantibodies. However, the detection rate varies depending on disease activity and organ involvement: Active SLE with renal involvement > 95%, active SLE without renal involvement > 50–70%, inactive SLE < 40%. However, the absence of dsDNA does not rule out an active flare of SLE (11,13,7,2,12).

2. Sm (Smith Antigen): Autoantibodies against Sm are highly specific and occur in approximately 10–15% of patients with SLE. They are positively associated with severe organ manifestations, such as CNS, kidney, or skin involvement. They are considered a prognostic marker, and their detection is one of the eleven ACR criteria. Sm-positive sera often also react with the Sm/RNP spot triplet, which contains epitopes of Sm in addition to the major antigen RNP (11,7,2,12,4,6).

3. Sm/RNP (Smith Antigen/Ribonucleoprotein): Autoantibodies against Sm/RNP are found in approximately 30–40% of patients with SLE, approximately 95% of patients with MCTD, and 10–15% of patients with scleroderma. In the absence of Sm and dsDNA autoantibodies, autoantibodies against RNP have a very high sensitivity of nearly 100% for MCTD. The absence of RNP autoantibodies therefore rules out MCTD (11,13,7,2,4,6).

4. PCNA (Proliferating Cell Nuclear Antigen): Autoantibodies against PCNA (identical to autoantibodies against cyclin) occur in approximately 3–7% of patients with SLE. Patients with PCNA-positive SLE frequently exhibit renal or CNS involvement as well as thrombocytopenia (11,13,7,2).

5. P0 (Ribosomal Phosphoprotein): P0 is a ribosomal phosphoprotein and the major component of the 60S subunit of the ribosomal complex. Autoantibodies against P0 occur in approximately 6–46% of patients with SLE, even when dsDNA and Sm autoantibodies are not detectable. Autoantibodies against P0 are highly specific and are primarily associated with SLE patients in the active phase with renal involvement (7,2,4,9).

6. SS-A 52 (Sjögren's syndrome antigen A): Autoantibodies against SS-A 52 are frequently found in patients with myositis and play a role in the pathogenesis of congenital heart block as well as cutaneous lupus manifestations. Autoantibodies against SS-A 52 are less specific for Sjögren's syndrome (SjS) and systemic lupus erythematosus (SLE) than autoantibodies against SS-A 60. The concurrent presence of SS-A 52, SS-A 60, and SS-B autoantibodies is associated with congenital heart block (7,2,4,6,1,3).

7. SS-A 60 (Sjögren's syndrome antigen A): Autoantibodies against SS-A are present in approximately 96% of patients with primary Sjögren's syndrome (SjS) and approximately 80% of patients with secondary SjS, approximately 24–60% of patients with lupus erythematosus (LE), approximately 70–100% of patients with subacute cutaneous lupus erythematosus (SCLE), approximately 85% of patients with neonatal lupus erythematosus (NLE), and approximately 5–8% of patients with RA. The concurrent presence of SS-A 52, SS-A 60, and SS-B autoantibodies is associated with congenital heart block (11,7,2,4,6,1,3).

8. SS-B (Sjögren's syndrome antigen B): Autoantibodies against SS-B are found in approximately 70% of patients with primary SjS, approximately 50% of patients with secondary SjS, approximately 20% of patients with LE, approximately 50% of patients with subacute cutaneous lupus erythematosus (SCLE), and approximately 60% of patients with neonatal lupus erythematosus (NLE). If SS-B autoantibodies occur simultaneously with SS-A 52 and SS-A 60 autoantibodies in pregnant women, there is an increased risk of 1–2% for congenital heart block in the newborn (11,7,2,4,1,3).

9. Scl-70 (Topoisomerase I): Autoantibodies against Scl-70 occur in approximately 40% of patients with SSc. Generally, Scl-70-positive patients have more severe disease courses with a poorer prognosis than patients who are positive for centromere autoantibodies. There is an association with diffuse skin involvement and the occurrence of internal manifestations, e.g., affecting the lungs, heart, and kidneys (11,13,7,2,10).

10. CENP B (Centromere B Antigen): Autoantibodies against CENP B are present in approximately 64–95% of patients with CREST syndrome and in approximately 20–35% of patients with diffuse scleroderma. At over 95%, CENP B is the most common target antigen in patients with scleroderma. Patients with primary biliary cirrhosis (PBC) also have autoantibodies against the centromere in approximately 30% of cases. The incidence of interstitial pulmonary fibrosis and renal involvement is very low in centromere-positive scleroderma patients; however, organ manifestations such as pulmonary hypertension and gastrointestinal complications occur more frequently (7,2,4,10).

11. Jo-1 (histidyl-tRNA synthetase): Autoantibodies against Jo-1 are present in approximately 60% of patients with PM, approximately 13% of patients with DM, and approximately 43% of patients with PM/DM overlap syndrome. The diagnostic specificity is nearly 100%. There is an association with SS-A 52 autoantibodies. Jo-1-positive myositis patients have a severe course of the disease, frequent flare-ups, and a poor prognosis. Interstitial lung involvement is present in over 70% of cases. The majority of myositis patients with Jo-1 autoantibodies exhibit symptoms of anti-synthetase syndrome. With successful treatment or during remission, the Jo-1 autoantibodies disappear (11,13,7,2,5).

12. PM/Scl (Exoribonuclease): Autoantibodies against PM/Scl exoribonucleases are present in approximately 24–55% of patients with polymyositis/scleroderma overlap syndrome, approximately 8–12% of patients with PM/DM, and approximately 1–16% of patients with scleroderma. In addition to myositis, PM/Scl is a prognostic marker for arthritis in approximately 89% of cases, Raynaud's symptoms in approximately 79%, interstitial lung disease in approximately 44% of cases, and scleromyositis in childhood. PM/Scl autoantibodies usually occur in isolation and not in combination with other markers of idiopathic myositis or scleroderma (13,7,2,10,8).

13. DFS70 (Dense Fine Speckled 70): Autoantibodies against DFS70 may correlate with a dense, fine-speckled nuclear pattern in HEp2-IFT. The detection of DFS70 autoantibodies can thus help explain observed patterns in HEp2-IFT, particularly when no other autoantibodies are detectable (15,16,17,18).

Performance Data

Agreement between the reactivity of the ANA plus ViraChip® IgG and the ANA ViraChip® IgG.

The following tables (Tables 1–13) show the agreement between the reactivity of the ANA plus ViraChip® IgG and the reactivity of the reference method, ANA ViraChip® IgG. A total of 242 sera that tested positive in the HEp-2 IFT were used.

dsDNA	ANA ViraChip®	
ANA plus ViraChip®	Positive	Negative
Positive	**23**	**1**
Negative	**4**	**214**
	Correlation: 98%	

Table 1: Agreement between the reactivity of the dsDNA spot duplet on the ANA plus ViraChip® IgG and the dsDNA spot triplet on the ANA ViraChip® IgG.

SS-B	ANA ViraChip®	
ANA plus ViraChip®	Positive	Negative
Positive	**39**	**1**
Negative	**1**	**201**
	Correlation: 99%	

Table 8: Agreement between the reactivity of the SS-B spot duplet on the ANA plus ViraChip® IgG and the SS-B spot triplet on the ANA ViraChip® IgG.

© Copyright VIRAMED Biotech AG January 2024 0122/0950

VIRAMED BIOTECH AG • Behringstraße 11 • 82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
viramed@viramed.de • www.viramed.de

PCNA	ANA ViraChip®	
ANA plus ViraChip®	Positive	Negative
Positive	**0**	**0**
Negative	**0**	**242**
	Correlation: 100%	

Table 2: Agreement between the reactivity of the PCNA spot duplet on the ANA plus ViraChip® IgG and the PCNA spot triplet on the ANA ViraChip® IgG.

P0	ANA ViraChip®	
ANA plus ViraChip®	Positive	Negative
Positive	**14**	**1**
Negative	**2**	**225**
	Correlation: 99%	

Table 3: Agreement between the reactivity of the P0 spot duplet on the ANA plus ViraChip® IgG and the P0 spot triplet on the ANA ViraChip® IgG.

SS-A 52	ANA ViraChip®	
ANA plus ViraChip®	Positive	Negative
Positive	**83**	**1**
Negative	**8**	**150**
	Correlation: 96%	

Table 4: Agreement between the reactivity of the SS-A 52 spot duplet on the ANA plus ViraChip® IgG and the SS-A 52 spot triplet on the ANA ViraChip® IgG.

SS-A 60	ANA ViraChip®	
ANA plus ViraChip®	Positive	Negative
Positive	**105**	**1**
Negative	**9**	**127**
	Correlation: 96%	

Table 5: Agreement between the reactivity of the SS-A 60 spot duplet on the ANA plus ViraChip® IgG and the SS-A 60 spot triplet on the ANA ViraChip® IgG.

Sm	ANA ViraChip®	
ANA plus ViraChip®	Positive	Negative

CENP B	ANA ViraChip®	
ANA plus ViraChip®	Positive	Negative
Positive	**14**	**0**
Negative	**0**	**228**
	Correlation: 100%	

Table 9: Comparison of the reactivity of the CENP B spot duplet on the ANA plus ViraChip® IgG with the CENP B spot triplet on the ANA ViraChip® IgG.

Scl-70	ANA ViraChip®	
ANA plus ViraChip®	Positive	Negative
Positive	**16**	**2**
Negative	**0**	**224**
	Correlation: 99%	

Table 10: Agreement between the reactivity of the Scl-70 spot duplet on the ANA plus ViraChip® IgG and the Scl-70 spot triplet on the ANA ViraChip® IgG.

PM/Scl	ANA ViraChip®	
ANA plus ViraChip®	Positive	Negative
Positive	**1**	**2**
Negative	**0**	**239**
	Correlation: 99%	

Table 11: Agreement between the reactivity of the PM/Scl spot duplet on the ANA plus ViraChip® IgG and the PM/Scl spot triplet on the ANA ViraChip® IgG.

Jo-1	ANA ViraChip®	
ANA plus ViraChip®	Positive	Negative
Positive	**7**	**0**
Negative	**0**	**235**
	Correlation: 100%	

Table 12: Comparison of the reactivity of the Jo-1 spot duplet on the ANA plus ViraChip® IgG with the Jo-1 spot triplet on the ANA ViraChip® IgG.

DFS70*	ANA ViraChip®	
ANA plus ViraChip®	Positive	Negative

© Copyright VIRAMED Biotech AG January 2024 0122/0950

VIRAMED BIOTECH AG • Behringstraße 11 • 82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
viramed@viramed.de • www.viramed.de

Positive	**30**	**8**
Negative	**1**	**203**
	Correlation: 96%	

Table 6: Agreement between the reactivity of the Sm spot duplet on the ANA plus ViraChip® IgG and the Sm spot triplet on the ANA ViraChip® IgG.

Sm/RNP	**ANA ViraChip®**	
ANA plus ViraChip®	Positive	Negative
Positive	**39**	**1**
Negative	**0**	**202**
	Correlation: 100%	

Table 7: Agreement between the reactivity of the Sm/RNP spot duplet on the ANA plus ViraChip® IgG and the Sm/RNP spot triplet on the ANA ViraChip® IgG.

Positive	**2**	**0**
Negative	**0**	**25**
	Correlation: 100%	

Table 13: Agreement between the reactivity of the DFS70 spot duplet on the ANA plus ViraChip® IgG and the DFS70 spot triplet on the ANA ViraChip® IgG.

*27 sera were used to determine the reactivity.

The following table shows the specificity of the individual antigens nucleosome and histone.

	IFT negative	
ANA plus ViraChip®	**Nucleosome**	**Histone**
Positive	**1***	**3***
Negative	**139**	**137**
	Correlation: 99%	**Correlation: 98%**

Table 14: Specificity of the individual antigens Nucleosome and Histone with the ANA plus ViraChip® IgG.
*Additional data regarding the sensitivity for Mi-2, Ku, nucleosomes, and histones are available upon request.

ANA Antigens	**Blood donor sera n = 141**	
	ANA plus ViraChip® IgG positive	**Specificity**
dsDNA	**0**	**100%**
Sm	**1**	**99%**
Sm/RNP	**0**	**100%**
PCNA	**1**	**99%**
P0	**1**	**99%**
SS-A 52	**0**	**100%**
SS-A 60	**0**	**100%**
SS-B	**0**	**100%**
Scl-70	**1**	**99%**

© Copyright VIRAMED Biotech AG January 2024 0122/0950

VIRAMED BIOTECH AG • Behringstraße 11 • 82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
viramed@viramed.de • www.viramed.de

CENP B	**0**	**100%**
Jo-1	**1**	**99%**
PM/Scl	**1**	**99%**
DFS70	**2**	**99%**
Nucleosome	**0**	**100%**
Histone	**2**	**99%**
Ku	**1**	**99%**
Wed-2	**0**	**100%**

Table 15: Specificity of autoantibodies using the ANA plus ViraChip® IgG in European blood donors.

User Requirements

1. This test must only be performed in a specialized laboratory that possesses the appropriate laboratory equipment (e.g., in accordance with "Good Laboratory Practice," GLP) and any necessary regulatory approvals for handling the samples to be tested.
2. To obtain an accurate test result, the instructions for use and GLP must be followed.
3. This test must only be performed by qualified personnel who have been trained in the test procedure.

Storage and Shelf Life of the Reagents

1. ViraChip® Microarrays: When sealed in the original packaging, they are stable at 2–8°C until the expiration date.
2. Wash Buffer Concentrate, 10x: Stable at 2–8°C until the expiration date.
3. Washing Buffer Working Solution: Stable for approximately 2 weeks at 2–8°C. For longer storage, the working solution may be aliquoted and frozen at -20°C.
4. Sample buffer: Store at 2–8°C until the expiration date.
5. Conjugate solution: Store at 2–8°C until the expiration date.
6. Chromogen/substrate solution: Store at 2–8°C until the expiration date. Protect from light!
7. Once the reagents and kit components have been opened for the first time, the shelf life is generally maintained until the specified expiration date when used properly ("Good Laboratory Practice") and stored in the original packaging under the conditions indicated on the label.

Precautions / Safety Measures

1. All human serum components have been tested for HCV, HIV-1 and HIV-2 antibodies, and HBs antigen, and found to be negative. Nevertheless, all human kit components, as well as patient samples, should be considered potentially infectious and handled with appropriate precautions. When working with potentially hazardous or infectious materials, the applicable national and international laws, ordinances, and regulations must be observed. This also applies to the storage and disposal of the chemicals and reagents used.
2. Safety precautions for working with hazardous and biological substances must be followed in accordance with the currently valid country-specific guidelines for laboratories. These precautions include, among others:
- Do not pipette with your mouth.
- Wear disposable gloves and safety goggles or a face shield.
- Do not eat, drink, or smoke at the workplace.

3. Avoid contact between the skin and mucous membranes and the Chromogen/substrate solution. In case of contact with the skin or eyes, rinse immediately with plenty of water.
4. Samples and all potentially contaminated materials, as well as liquid infectious waste, must be decontaminated in accordance with generally accepted laboratory guidelines, e.g., by autoclaving for at least 20 minutes at 121°C. To dispose of liquid waste, it can be mixed, for example, with sodium hypochlorite in a volume ratio such that the final mixture contains 1% sodium hypochlorite. Allow it to act for 30 minutes to ensure complete disinfection.
5. Information on potential hazards, first aid measures, actions to take in case of accidental release of large quantities, handling and storage, personal protective equipment, disposal instructions, and toxicological data is documented in the safety data sheets.
6. Prevent dust and other contaminants from entering the wells of the MTP, as this may result in invalid results.

Collection, Handling, and Storage of the Patient Sample

1. The **ANA plus ViraChip® IgG Test Kit** must be performed using human serum as specimen.
2. Specimen collection should be performed by healthcare professionals from whole blood in accordance with applicable standards (14).
3. Samples that have been heat-treated, or that are hemolyzed, icteric, or lipemic, may lead to invalid results.
4. Samples must not be microbiologically contaminated.
5. As a general rule, undiluted samples can be stored for up to 5 days at 2–8°C . For storage of up to 24 months, samples must be frozen in aliquots at a temperature of at least -20°C.
6. Before beginning the test, samples should be brought to room temperature. After thawing, mix the samples gently and, if necessary, remove visible particles by centrifugation.
7. Avoid repeated freezing and thawing of the samples.

Limitations of the Test Method

1. A positive test result for a given patient sample should be interpreted as a symptom. Interpretation and evaluation of test results may only be performed by qualified medical personnel after assessing all relevant data (13).
2. A negative test result does not rule out exposure to the pathogen or infection.
3. The detection of specific antibodies may yield different results when using different tests or tests from different manufacturers due to variations in sensitivity, specificity, and testing methods.
4. In cases of immunosuppression, the interpretability of the results is limited (13).
5. Medications and immunoglobulin administration can trigger nonspecific antibody reactions (13).

© Copyright VIRAMED Biotech AG January 2024 0122/0950

VIRAMED BIOTECH AG • Behringstraße 11 • 82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
viramed@viramed.de • www.viramed.de

6. *In* vitro diagnostic reagents must not be used beyond their stated expiration date, as reliable results can no longer be guaranteed.
7. Strict adherence to the operating instructions is essential for accurate test results. Inadequate performance of the washing steps can lead to incorrect results.
8. The use of pooled samples, in which multiple sera are tested together, is not permitted, as such samples may compromise the test's sensitivity and specificity.

Notes on Equipment and Software

1. When performing the test automatically, work steps validated by Viramed Biotech AG for the respective processor type must be programmed and used.
2. When using processor-specific consumables, the corresponding configurations must be approved by Viramed Biotech AG in accordance with the manufacturer's instructions.
3. The device and software configuration provided by Viramed Biotech AG must not be modified. Any modification may cause incorrect results.
4. Only device-specific software may be used. Changes to configuration files may only be made by Viramed Biotech AG.
5. Only systems approved by Viramed Biotech AG may be used as measuring devices.
6. The ViraChip® microarrays must be analyzed exclusively using the ViraChip® Software. Manual or visual analysis is not possible.

References

1. BRUCATO, A et al.: Risk of congenital complete heart block in newborns of mothers with anti-Ro/SSA antibodies detected by counterimmunoelectrophoresis: a prospective study of 100 women, Arthritis Rheum, 2001
2. CONRAD, K et al.: Autoantikörper bei systemischen Autoimmunerkrankungen, Pabst Science Publishers, 3 Auflage, 2006
3. GORDON, P et al.: Anti-52 kDa Ro; anti-60 kDa Ro and anti-La antibody profiles in neonatal lupus, J Rheumatol, 2004
4. HOFFMANN, MH et al.: Nucleic acid-associated autoantigens: Pathogenic involvement and therapeutical potential, J Autoimmun, 2010
5. KAO, AH et al.: Anti-Signal recognition particle autoantibody in patients with and patients without idiopathic inflammatory myopathy, Journal of experimental Medicine, 2005
6. MIGLIORINI, P et al.: Anti-Sm and Anti-RNP antibodies, Autoimmunity, 2005
7. VON MÜHLEN, CA & TAN, EM: Autoantibodies in the diagnosis of systemic rheumatic diseases, Seminars in Arthritis and Rheumatism, 1995
8. REICHLIN, M et al.: Antibodies to a nuclear/nucleolar antigen in patients with polymyositis overlap syndromes, Journal of Clinical Immunology, 1984
9. REICHLIN, M: Serological correlations with nephritis in systemic lupus erythematosus, Clin Immunol, 2005
10. STEEN, VD: Autoantibodies in systemic sclerosis, Seminars in Arthritis and Rheumatism, 2005
11. TAN, EM: Antinuclear Antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology, Adv Immunol, 1989
12. HOCHBERG MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus, Arthritis Rheum, 1997
13. THOMAS, L: Labor und Diagnose, Med Verlagsgesellschaft Marburg, 2012
14. TUCK, MK et al.: Standard Operating Procedures for Serum and Plasma Collection, J Proteome Res, 2009
15. HEROLD, M et al.: ICAP – ein Versuch zur einheitlichen Beschreibung der Fluoreszenzmuster von antizellulären Antikörpern auf HEp-2-Zellen, J Lab Med, 2017
16. BENTOW, C et al.: Recognition of the dense fine speckled (DFS) pattern remains challenging: results from an international internet-based survey, Auto Immun Highlights, 2016
17. CARTER, JB et al.: Recognition and relevance of anti-DFS70 autoantibodies in routine antinuclear autoantibodies testing at a community hospital, Frontiers in Medicine, 2018
18. CONRAD, K et al.: The Clinical Relevance of Anti-DFS70 Autoantibodies, Clinic Rev Allerg Immunol, 2017

Legend

	Manufacturer		Order Number
	Follow the instructions for use		Use by / Best-by date
	*In-vitro* diagnostic		Temperature Limit (Storage)
	Lot number		Positive control serum
	Sufficient for 96 tests		Negative control serum

© Copyright VIRAMED Biotech AG January 2024 0122/0950

VIRAMED BIOTECH AG • Behringstraße 11 • 82152 Planegg • Germany • HRB 134 839 • Tel.: +49 - (0) 89 - 89 93 36 • Fax: +49 - (0) 89 - 859 99 49
viramed@viramed.de • www.viramed.de