LABORATORY • DIAGNOSTICS

Bordetella pertussis ViraStripe® IgA Test Kit

Stripe-Immunoblot for the qualitative detection of **IgA** antibodies against specific **Bordetella species** antigens in human serum.

The **Bordetella pertussis ViraStripe® IgA Test Kit** is an immunoblot based on an enzyme-immunoassay in the line/stripe format carrying the following purified Bordetella specific antigens: filamentous hemagglutinin **(FHA)** and pertussis toxin **(PT)**.

Reactivity of the bands **PT** and **FHA** have been calibrated according to **WHO standard sera** and thus allow correlating the data measured to International Units per millilitre (3).

Principle of the assay

During the serum incubation step Bordetella specific IgA antibodies bind to the immobilised antigens on the test strip. During the conjugate reaction, AP-conjugate binds to the antigen-antibody complex. The alkaline phosphatase converts the chromogen/substrate and thus, stains the antigen-antibody complex on the test strip purple. The washing procedures following serum, conjugate and chromogen/substrate incubation steps remove unbound antibodies and reagents.

The green separation line divides the test strip into a control section and an analytical section. The control section contains the **negative control band**, the **serum control, three conjugate controls** (IgG, IgA, IgM) and the **cut off control**.
The test strip code for Bordetella pertussis ViraStripe® IgA test strips is **PA**. Test strips are numbered from **01** to **50.** The analytical section contains the Bordetella specific antigens.

Order No.:	**V-BPSAOK**	Order No.:	**V-BPSADK (Deca Kit)**
Kit size:	**1x 50 test strips**	Kit size:	**10x 50 test strips**
Specimen:	**20 µl serum**	Specimen:	**20 µl serum**
Time for testing:	**approx. 90 minutes**	Time for testing:	**approx. 90 minutes**

Materials provided

1x or 10x 50 test strips	**Bordetella pertussis ViraStripe® Antigen Strips (IgA)** Test strips including a control section and Bordetella specific antigens in the analytical section, ready to use	(Prod. No.: V-BPSAAS)
1x or 10x 9 ml	**ViraStripe® / ViraBlot® AP-Anti-Human IgA Conjugate** Concentrate, goat	(Order No.: V-UVNAKI)
1x or 10x 100 ml	**ViraStripe® / ViraBlot® Diluent / Wash Buffer** 10x concentrate	(Order No.: V-UVNUWP)
1x or 10x 5 g	**ViraStripe® / ViraBlot® Diluent / Wash Powder**	(Order No.: V-UVNUMP)
1x or 10x 90 ml	**ViraStripe® / ViraBlot® Chromogen / Substrate Solution** Ready to use	(Order No.: V-UVNUCS)
1 or 10 copies	**Evaluation Protocol** for **Bordetella pertussis ViraStripe® IgA Test Kit**	

Additionally available

330 µl	**Bordetella pertussis ViraStripe® IgA Positive Control** Human, ready to use	(Order No.: V-BPSAPK)
330 µl	**Bordetella pertussis ViraStripe® IgG,A,M Negative Control** Human, ready to use	(Order No.: V-BPSPNK)
50 copies	**Bordetella pertussis ViraStripe® IgA evaluation protocols** for automated interpretation with ViraScan® software	(Order No.: V-BPSAEP)

Preparation of reagents and patient samples

Bring all reagents to room temperature (20-25°C) prior to use. Information about stability can be found on page 5.

Diluent / Wash Buffer Working Dilution:	Dilute **Diluent / Wash Buffer Concentrate 1:10** with distilled or deionised water (100 ml concentrate + 900 ml water). Add Diluent / Wash Powder completely and stir well until all powder is dissolved. If needed, place onto a magnetic stirrer for 10-15 minutes. The pH value should be around pH 7.5 at 20°C.
Antigen Strips:	Carefully separate the required number of test strips by use of **forceps** at the **label** and place the test strips in the prepared incubation tray (see assay procedure, step 2). Use test strips directly after removing from packing. Do not touch test strips by hand. Return unused test strips directly into the original packing, seal well and store at 2-8°C.
Patient samples:	Use **20 µl patient serum** undiluted per test strip.
Controls:	Use **100 µl** of **Positive Control** or **100 µl** of **Negative Control** undiluted per test strip respectively.
Conjugate Working Dilution:	Prepare **Conjugate Concentrate 1:10** with Diluent / Wash Buffer Working Dilution (see table 1). Prepare freshly prior to each test run. Do not store for further use.
Chromogen / Substrate Solution:	Ready to use.

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Preparation of Conjugate Working Dilution IgA

Number of strips	Diluent / Wash Buffer Working Dilution		Conjugate Concentrate	Final volume
1	1.35 ml	+	0.15 ml	1.5 ml
2	2.70 ml	+	0.30 ml	3.0 ml
3	4.05 ml	+	0.45 ml	4.5 ml
4	5.40 ml	+	0.60 ml	6.0 ml
5	6.75 ml	+	0.75 ml	7.5 ml
6	8.10 ml	+	0.90 ml	9.0 ml
7	9.45 ml	+	1.05 ml	10.5 ml
8	10.80 ml	+	1.20 ml	12.0 ml
9	12.15 ml	+	1.35 ml	13.5 ml
10	13.50 ml	+	1.50 ml	15.0 ml
11	14.85 ml	+	1.65 ml	16.5 ml
12	16.20 ml	+	1.80 ml	18.0 ml
13	17.55 ml	+	1.95 ml	19.5 ml
14	18.90 ml	+	2.10 ml	21.0 ml
15	20.25 ml	+	2.25 ml	22.5 ml
16	21.60 ml	+	2.40 ml	24.0 ml
17	22.95 ml	+	2.55 ml	25.5 ml
18	24.30 ml	+	2.70 ml	27.0 ml
19	25.65 ml	+	2.85 ml	28.5 ml
20	27.00 ml	+	3.00 ml	30.0 ml
21	28.35 ml	+	3.15 ml	31.5 ml
22	29.70 ml	+	3.30 ml	33.0 ml
23	31.05 ml	+	3.45 ml	34.5 ml
24	32.40 ml	+	3.60 ml	36.0 ml
25	33.75 ml	+	3.75 ml	37.5 ml

Number of strips	Diluent / Wash Buffer Working Dilution		Conjugate Concentrate	Final volume
26	35.10 ml	+	3.90 ml	39.0 ml
27	36.45 ml	+	4.05 ml	40.5 ml
28	37.80 ml	+	4.20 ml	42.0 ml
29	39.15 ml	+	4.35 ml	43.5 ml
30	40.50 ml	+	4.50 ml	45.0 ml
31	41.85 ml	+	4.65 ml	46.5 ml
32	43.20 ml	+	4.80 ml	48.0 ml
33	44.55 ml	+	4.95 ml	49.5 ml
34	45.90 ml	+	5.10 ml	51.0 ml
35	47.25 ml	+	5.25 ml	52.5 ml
36	48.60 ml	+	5.40 ml	54.0 ml
37	49.95 ml	+	5.55 ml	55.5 ml
38	51.30 ml	+	5.70 ml	57.0 ml
39	52.65 ml	+	5.85 ml	58.5 ml
40	54.00 ml	+	6.00 ml	60.0 ml
41	55.35 ml	+	6.15 ml	61.5 ml
42	56.70 ml	+	6.30 ml	63.0 ml
43	58.05 ml	+	6.45 ml	64.5 ml
44	59.40 ml	+	6.60 ml	66.0 ml
45	60.75 ml	+	6.75 ml	67.5 ml
46	62.10 ml	+	6.90 ml	69.0 ml
47	63.45 ml	+	7.05 ml	70.5 ml
48	64.80 ml	+	7.20 ml	72.0 ml
49	66.15 ml	+	7.35 ml	73.5 ml
50	67.50 ml	+	7.50 ml	75.0 ml

Table 1: **1:10** dilution of conjugate concentrate with Diluent / Wash Buffer Working Dilution

Assay procedure

1. **Rinse incubation tray channels once with 1.5 ml Diluent / Wash Buffer Working Dilution, decant the liquid**	Mark the trays with water-resistant pen. Rinsing removes dust particles.
2. **Place the needed amount of test strips into the incubation tray - one test strip per channel**	For each patient serum and each control, carefully separate one test strip by use of forceps at the label and place them into the incubation tray channels. **The side showing the green separation line and the label must face up.**
3. **Add 1.5 ml Diluent / Wash Buffer Working Dilution and incubate by rocking for 5 minutes at room temperature (RT)**	Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min. Avoid spilling of liquid. **Do not decant the liquid after incubation.**
4. **Add 20 µl of each patient serum or 100 µl of each control**	Add patient sera and controls directly onto the labelled end of the test strips while the 2D rocker is running or make sure to tilt the incubation tray after adding each serum.
5. **Incubate by rocking for 30 minutes at RT**	Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.
6. **Decant the liquid**	Carefully tap the incubation tray on absorbent paper to remove the remaining liquid. **Test strips adhere to the incubation tray when liquid is decanted.**
7. **3 x washing:** **- add 1.5 ml Diluent / Wash Buffer Working Dilution** **- incubate by rocking for 5 minutes at RT** **- decant the liquid**	Wash on the 2D rocker. Carefully tap the incubation tray on absorbent paper to remove the remaining liquid.
8. **Add 1.5 ml fresh Conjugate Working Dilution**	Make sure the test strips are completely covered with Conjugate Working Dilution
9. **Incubate by rocking for 15 minutes at RT**	Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.
10. **Decant the liquid**	Carefully tap the incubation tray on absorbent paper.
11. **3 x washing as in step 7**	Wash on the 2D rocker.
12. **Add 1.5 ml distilled or deionised water and incubate by rocking for 1 minute at RT**	Make sure the test strips are completely covered with liquid. Use a 2D rocker with a rocking frequency of approx. 40/min.
13. **Decant the liquid**	Carefully tap the incubation tray on absorbent paper.
14. **Add 1.5 ml Chromogen / Substrate Solution**	Make sure the test strips are completely covered with liquid.
15. **Incubate by rocking at RT** **Bordetella pertussis ViraStripe® IgA: approx. 5 to 15 minutes**	Stop the reaction as soon as the cut off control becomes visible. **The Cut off control is located in the test strip control section. Caution:** Prolonged incubation causes background staining.
16. **Stop the reaction by decanting the liquid**	Carefully tap the incubation tray on absorbent paper.
17. **Wash 3 x with 1.5 ml distilled or deionised water**	Wash without incubation time.
18. **Dry test strips for interpretation**	Carefully tap the incubation tray on absorbent paper to remove the remaining liquid. Place wet test strips with forceps on unbleached absorbent paper and allow to air dry before interpretation.

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Assay interpretation

1. Evaluation protocol: Record data on the evaluation protocol. Glue the test strips on the evaluation protocol. Place the green separation line of the test strips exactly onto the separation line printed on the evaluation protocol.

2. Validity of test strips: A test strip is considered as valid if the following bands are visible:
   - The **serum control**.
   - The **conjugate control** of the conjugate class being used. If more than one of the three conjugate controls become visible, the strongest band must indicate the appropriate conjugate class.
   - The **cut off control**.

   and if the following band is **not** visible:
   - The **negative control band**.

   Do not assess invalid test strips!

3. Assignment of antigen bands: The green separation line of the test strips indicates position and orientation for the assignment of bands with the bandlocator on the evaluation protocol. Assign bands and record results according to 4.

4. Assessment of bands: According to quality laboratory guidelines, the use of a cut off control for each run is recommended (4). **The cut off control of the Bordetella pertussis ViraStripe® IgA is located in the control section on each test strip.** The intensity of the cut off control indicates the threshold of which bands are being assessed:

   A band is considered as **distinct** if its intensity is **equal** to or **higher** than the intensity of the cut off control. Mark bands with **X** in the evaluation protocol appropriately.

   A band is considered as **weak** if its intensity is **lower** than the intensity of the cut off control. Mark bands with **(X)** in the evaluation protocol appropriately.
   Caution: A band is not assessed if barely visible**.** A band is not assessed if **not present.**

5. Interpretation of patient bands: Patient bands have to be considered as symptoms of the disease. A final clinical diagnosis should always be made considering anamnesis, clinical manifestations and laboratory data.
   Band **PT (28 kD)** is considered as **highly specific** for Bordetella pertussis and band **FHA (220 kD)** for Bordetella species.

IgA Interpretation criteria

General note: Distinct bands must have a minimum intensity (≥ cut off), which has to be determined by the cut off control. Weak bands have an intensity < cut off control. The cut off control is located in the control section of each test strip.

Identified bands	Result	Interpretation
Distinct PT band	**Positive**	PT-IgA antibodies against **Bordetella pertussis** detectable. This band pattern corresponds to a PT-IgA antibody concentration of about 12 IU/ml or greater. Following RKI guidelines this correlates with a once clearly increased IgA value (2), which provides an indication for recent contact with Bordetella pertussis (1). This band pattern might also be associated with a vaccine titer, especially if vaccination has recently occurred. However, IgA antibodies are rarely seen after vaccination (6,7).
Weak PT band	**Equivocal**	Low PT-IgA antibody amount against **Bordetella pertussis** detectable. If an infection is further suspected, check for a second patient sample after 2-4 weeks (1).
No PT band	**Negative**	No PT-IgA antibodies against **Bordetella pertussis** detectable. If an infection is further suspected, check a second patient sample after 2-4 weeks (1).
Distinct FHA band	**Positive**	FHA-IgA antibodies against **Bordetella species** detectable. Suspicion of recent contact with Bordetella species (8).
Weak FHA band	**Equivocal**	Low FHA-IgA antibody amount against **Bordetella species** detectable. If an infection is further suspected, check a second patient sample after 2-4 weeks (1).
No FHA band	**Negative**	No FHA-IgA antibodies against **Bordetella species** detectable. If an infection is further suspected, check a second patient sample after 2-4 weeks (1).

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IgG / IgA interpretation criteria of the pertussis toxin (PT) bands

PT-100 IgG	PT IgG	PT IgA	PT assessment	Interpretation of IgG und IgA results combination
x	**x**	**x** (x) ∅	**Positive**	Indication for recent contact with Bordetella pertussis (1,2,5). This band pattern might also be associated with a vaccine titer, especially if vaccination has occurred less than 12 months ago. However, IgA antibodies are rarely seen after vaccination (6,7).
(x) ∅	**x** (x) ∅	**x**	**Positive**	
(x)	**x**	(x) ∅	**Positive**	Suspicion of recent contact with Bordetella pertussis. If an infection is further suspected, check a second patient sample after 2-4 weeks (1).
∅	**x**	(x) ∅	**Positive**	Suspicion of recent contact with Bordetella pertussis or vaccine titer. A very early infection may not be excluded. If an infection is further suspected, check a second patient sample after 2-4 weeks (1).
(x) ∅	(x) ∅	**(x)**	**Equivocal**	If an infection is further suspected, check a second patient sample after 2-4 weeks (1).
∅	(x) ∅	∅	**Negative**	No indication for an infection with Bordetella pertussis or a vaccine titer.

Table 2: Results of PT bands of Bordetella pertussis ViraStripe® IgG, IgA and their laboratory diagnostic evaluation.
Legend: x = distinct band; (x) = weak band; ∅ = not existing band; Key bands of the band constellations are marked in grey.

IgG / IgA interpretation criteria of the filamentous hemagglutinin (FHA) bands

IgG FHA	IgA FHA	FHA assessment	Interpretation of IgG und IgA results combination
x (x) ∅	**x**	**Positive**	Suspicion of recent contact with Bordetella species (8).
x	(x) ∅	**Positive**	Suspicion of vaccine titer or past infection (1).
(x) ∅	**(x)**	**Equivocal**	If an infection is further suspected, check a second patient sample after 2-4 weeks (1).
(x) ∅	∅	**Negative**	No indication for an infection with Bordetella pertussis or a vaccine titer.

Table 3: Results of FHA bands of Bordetella pertussis ViraStripe® IgG, IgA and their laboratory diagnostic evaluation.
Legend: x = distinct band; (x) = weak band; ∅ = not existing band; Key bands of the band constellations are marked in grey.

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IgA test strip

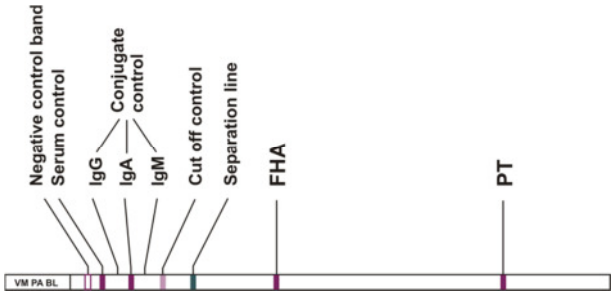

Figure 1: Schematic drawing of a Bordetella pertussis ViraStripe® IgA test strip in full scale.

Nomenclature and description of Bordetella bands from literature

Antigen:	**Comment:**
FHA (220 kD) Filamentous Hemagglutinin	Specific for Bordetella species: IgG antibodies against FHA appear in 80-90% of infected patients, whereas IgA antibodies appear in 50-60% of infected patients (9). Antibodies to FHA are developed after vaccination and after infection with Bordetella pertussis or Bordetella parapertussis.
PT (28 kD) Pertussis Toxin	Highly specific for Bordetella pertussis. IgG antibodies against PT appear in more than 90% of infected patients, whereas IgA antibodies appear in 40-50% of infected patients (9). Antibodies against PT are developed after vaccination and after infection with Bordetella pertussis but not after infection with Bordetella parapertussis. The PT-IgA band is calibrated with International WHO standards and when present in cut off intensity correlates with a value of about 12 IU/ml (3,12). Following RKI guidelines, a once clearly increased IgA value presents supportive evidence for a recent contact with Bordetella pertussis (1). This value corresponds to a distinct PT-IgA band.

Diagnostic significance of antibodies against Bordetella pertussis

1. IgG antibodies appear 15-20 days after beginning of the disease (Stadium convulsivum). They are not detectable in the early stage of the infection (13). IgG antibodies can persist more than 10 years, but at least 6 months after beginning of the disease (13,14). Therefore patients in the second (Stadium convulsivum) or third (Stadium decrementi) stage of the disease are mostly positive for IgG antibodies. Antibody titers steadily decrease in convalescence (15). Infants can acquire maternal IgG antibodies diaplacentally (9, 16).
As proof for an infection with Bordetella pertussis, the Robert Koch Institute (RKI) recommends for serological testing methods a once clearly increased value of at least 100 IU/ml for PT-IgG antibodies (1,2). This value correlates with a distinct PT-100 band on the Bordetella pertussis ViraStripe® IgG. Serological indication for recent contact with Bordetella pertussis is possible, if vaccination has occurred more than 12 months ago (1).

2. IgM antibodies usually appear 8-15 days after beginning of the disease (13) and reach their highest concentration after approx. 8-10 weeks (15). IgM antibodies are detectable in more than 90% of infected patients between the days 20 and 50 after beginning of the disease. IgM titers may be elevated after vaccination (17). In singular cases IgM antibodies may appear only weak, delayed or not at all in infants and adults (14).

3. IgA antibodies are nearly exclusively detectable after natural infection and only in very rare cases after vaccination (6,7). IgA antibodies reach their highest concentration approx. 8-10 weeks after beginning of the disease (15). IgA antibodies are generally not longer detectable than 6 months after infection (13). In the first months of life infants do not - or only in a low range – develop IgA antibodies. Therefore infants should be checked for IgM antibodies (17).
There are indications for persisting of Bordetella pertussis specific IgA antibodies in the population caused by subclinical infections (17).
As proof for an infection with Bordetella pertussis, the Robert Koch Institute (RKI) recommends for serological testing methods a once clearly increased value of at least 12 IU/ml for PT-IgA antibodies (1,2). This value correlates with a distinct PT band on the Bordetella pertussis ViraStripe® IgA.

4. Detection of antibodies against pertussis toxin (PT) is specific for Bordetella pertussis (9,15,16,18).

5. Medication and immunoglobulin therapy can cause unspecific antibody reactions (19).

6. Cross reactions with FHA are known for infections with M. pneumoniae, C. pneumoniae und other bacteria (7).

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IgA performance data

Analysis of sensitivity and specificity:

Sensitivity of the Bordetella pertussis ViraStripe® IgA Test Kit has been determined by analysing WHO standards, containing a defined amount of anti-PT and anti FHA antibodies, measured in International Units per millilitre (IU/ml) (3).

The **PT band** shows the following reactivity: a distinct band starts at about 12 IU/ml, based on anti-PT IgA antibodies.
The **FHA band** shows the following reactivity: a distinct band starts at about 20 IU/ml, based on anti-FHA IgA antibodies. A weak band starts at about 10 IU/ml.

Reactivity of the Bordetella pertussis ViraStripe® IgA Test Kit has been determined by analysing 145 unselected blood donors.

Bordetella pertussis ViraStripe® IgA	Positive with a PT band, % (n)	Positive with a FHA band, % (n)
Blood donors **(n= 145)**	**2% (3)**	**29% (42)**

Table 4: Blood donor analysis with Bordetella pertussis ViraStripe® IgA Test Kit

Scientific studies demonstrate the presence of anti-PT IgA antibodies in blood donor sera in 2% of all cases, showing at least the "minimal level of quantitation" (12 IU/ml) (12). This level correlates to a distinct Bordetella pertussis ViraStripe® IgA **PT band**. Analysis of 145 unselected blood donor sera with the Bordetella pertussis ViraStripe® IgA Test Kit shows as well in 2% of all cases a positive result with the PT band (see table 4).

Reference studies demonstrate the presence of anti-FHA IgA antibodies in blood donor sera in 58% of all cases, showing at least the "minimal level of quantitation" (10 IU/ml) (12). Analysis of 145 unselected blood donor sera with the Bordetella pertussis ViraStripe® IgA Test Kit shows in 29% of all cases an at least distinct **FHA band** (20 IU/ml; see table 4).

Warnings and precautions

1. All human serum components were tested for HCV, HIV1,2 antibodies and HBs antigens and found to be negative. Nevertheless, all human kit components as well as the patient samples should be considered as potentially infectious and handled according to safety precautions. While working with potentially infectious/hazardous materials, all national and international rules, regulations, guidelines and laws must be taken into account. This also applies to storage and disposal of chemicals and reagents being used.
2. While working with hazardous or toxic substances/ biological agents precautions have to be applied following national biosafety regulations. In general, biological and chemical agents should be handled according to „Good Laboratory Practice (GLP)" guidelines. Precautions among others are:
- Do not pipette by mouth.
- Wear disposable gloves and safety glasses while working.
- Do not eat, drink or smoke in the working area.

3. The chromogen/substrate solution contains BCIP and NBT. Avoid contact with skin and mucous membranes. In case of contact with skin and eyes wash immediately with large quantities of water.
4. Samples and all potentially contaminated materials must be decontaminated using validated laboratory techniques, e.g. by autoclaving 20 minutes at 121°C under humid conditions. Liquid disposals can be mixed with sodium hypochlorite to a final concentration of 1% sodium hypochlorite. Incubate 30 min for complete disinfection.
5. Please refer to material safety data sheets for detailed information on potential risks, first aid guidelines, accidental release measures, handling and storage recommendations, personal protective equipment, directions for disposal and indications to toxicology.

Storage and stability of reagents

1. Test strips: In closed bags stable until the expiration date if stored at 2-8°C.
2. Conjugate Concentrate: Stable until the expiration date if stored at 2-8°C.
3. Conjugate Working Dilution: Prepare freshly prior to each run. Do not store for further use.
4. Diluent / Wash Buffer Concentrate, 10x: Stable until the expiration date if stored at 2-8°C.
5. Diluent / Wash Buffer Working Dilution: Stable for 2 weeks if stored at 2-8°C. For longer storage, aliquot and freeze at -20°C.
6. Diluent / Wash Powder: Stable until the expiration date if stored at 2-8°C.
7. Chromogen / Substrate Solution: Stable until the expiration date if stored at 2-8°C. Avoid exposure to light!

Specimen indications

1. The **Bordetella pertussis ViraStripe® IgA Test Kit** must be used with human serum.
2. Only clear, non-hemolysed, non-microbially contaminated specimens must be used.
3. Using icteric, lipemic, hemolytic and/or heat-inactivated serum may lead to false results.
4. Normally, human serum can be stored up to 5 days at 2-8°C. Specimens may be stored at -20°C (or below) for long term storage.
5. Prior test processing, specimens should have reached room temperature. Mix specimens carefully after thawing. Precipitates in specimens can be removed by centrifugation.
6. Avoid multiple freeze and thaw cycles.

Limitation of the procedure

1. To ensure reliable results, follow carefully the Instruction for Use and "Good Laboratory Practice".
2. A positive result is based on elevated specific antibody titers and should be considered as a symptom. The correlation to a disease is only conditionally possible.
3. A negative result does not exclude a contact with the pathogen or the presence of a disease.
4. Adequately trained personnel only should perform the assay procedure.
5. The detection of specific antibodies can vary within different assays from different manufacturers and can lead to different results due to different sensitivity, specificity and assay methodologies.
6. Test strips showing a high background level should not be interpreted, especially if band intensities are lighter than the background level.
7. *In vitro* diagnostics must not be used beyond expiration date as reliable results may not be possible.
8. Efficient washing after each incubation step is essential for consistent results; insufficient washing may lead to false results.

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Symbols used

	Manufacturer	REF	Order Number
	Refer to Instructions for Use		Use by / Expiration Date
IVD	*In-Vitro* Diagnostic Medical Device		Temperature Limitation (Storage)
LOT	Test Kit Lot Number	CONTROL +	Positive Serum Control
Σ 50	Sufficient for 50 Tests	CONTROL –	Negative Serum Control
	Room Temperature in °C	CONTROL	Control
	User	DATE	Date
#	Serum Number	SUBSTRATE	Chromogen/Substrate Incubation Time in Minutes
PROTOCOL	Evaluation Protocol	No	Protocol Number

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