Bordetella pertussis ViraStripe® IgG Testkit

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Immunoassay for the detection of **IgG** antibodies against **Bordetella species** in human serum.

The **Bordetella pertussis ViraStripe® IgG** is an **immunoblot** which carries Bordetella species specific antigens: filamentous hemagglutinin (FHA) and pertussis toxin (PT). The control section of each strip includes a serum control, three conjugate controls and a cut off control.
Bordetella pertussis toxin is applied in two different concentrations on the Bordetella pertussis ViraStripe® IgG, intended to differentiate between a **recent contact** with Bordetella pertussis (a once clearly increased IgG value) and a **past infection** (1,2).
Reactivity of bands **PT**, **PT-100** and **FHA** have been calibrated according to **WHO standard sera** und thus allow to correlate results with data measured in International Units per Millilitre (5).

Bordetella ViraStripe® IgG Testkit 50 tests Prod. No. V-BPSGOK

Specimen: **20 µl serum**
Time for testing: **approx. 90 minutes**
Storage/Stability: **approx. 12 months at 2-8°C**

Components of the Kit

50 strips	**IgG-specific Antigen Strips,** ready to use nitrocellulose strips including a control section and Bordetella species specific antigens in an analytical section Strip code for IgG: PG, strips are numbered from 1 to 50	(Prod. No. V-BPSGAS)
9 ml	**IgG-specific AP-Anti-Human Conjugate,** goat anti-human, 10x concentrate, liquid	(Prod.No.: V-UVNGKI)
100 ml	**Sample Diluent/Wash Buffer,** 10x concentrate	(Prod. No. V-UVNUWP)
1 package	**Sample Diluent/Wash Powder** (5g)	(Prod. No. V-UVNUMP)
90 ml	**Chromogen/Substrate Solution,** ready to use	(Prod. No. V-UVNUCS)
1 piece	**Instructions for Use - Bordetella pertussis ViraStripe® IgG Testkit**	
2 pieces	**Protocol sheets - Bordetella pertussis ViraStripe® IgG Testkit**	

Additionally available

330 µl	Bordetella pertussis ViraStripe® **IgG positive control**, human serum, ready to use	(Prod. No.: V-BPSGPK)
330 µl	Bordetella pertussis ViraStripe® **IgG, IgA, IgM negative control**, human serum, ready to use	(Prod. No.: V-BPSPNK)
50 pieces	Bordetella pertussis ViraStripe® **black protocol sheets** for automatic interpretation with ViraScan® software	(Prod. No.: V-BPSGEP)

Storage and Stability of Reagents

Antigen Strips: Strips in closed bags are stable until expiration date when stored at 2-8°C. Use strips immediately after removing from package. Close bags with unused strips tightly.

Conjugate:
- 10x concentrate: Stable until expiration date if stored at 2-8°C.
- Conjugate Working Dilution**:** To be used in a single use**.**

Sample Diluent/Wash Buffer:
- 10x concentrate and Sample Diluent/Wash Powder: stable until expiration date if stored at 2-8°C.
- Buffer Working Dilution: 2 weeks usable if stored at 2-8°C. Freeze the Buffer Working Dilution at -20°C in aliquots for longer storage.

Chromogen/Substrate Solution: Stable until expiration date if stored at 2-8°C. Avoid exposure to light!

Preparation of Reagents and Specimen

Bring all components to room temperature (20-25°C) prior to use.

Antigen Strips:
Antigen Strips are connected at the end with each other. Carefully separate the required number of strips by use of forceps and place the strips in rinsed incubation tray channels (see Test procedure, step 2). Pick strips with forceps at the label only and do not touch by hand. Return unused strips into the package and store at 2-8°C.

Buffer Working Dilution:
To prepare the Buffer Working Dilution, dilute the Sample Diluent/Wash Buffer 10x concentrate 1:10 with distilled water (100 ml concentrate + 900 ml distilled water) and add the Diluent/Wash Powder **completely**. Stir well for 10-15min using a magnetic stirrer until all salt is dissolved. pH of Buffer Working Dilution must be 7.5 ± 0.1.

Conjugate Working Dilution:
Dilute the needed amount of Conjugate 10x concentrate 1:10 with Buffer Working Dilution according to table 1. Prepare Conjugate Working Dilution freshly prior to the first washing step (see Test procedure, step 7).

Chromogen/Substrate Solution: Ready to use.

Specimen:
Use **20 µl patient serum** undiluted per test.

Additionally available controls:
Use **100µl** of each **positive control** and **negative control** undiluted per test run.

Table 1: Conjugate Working Dilution

Number of strips	Buffer Working Dilution		Conjugate-concentrate	Conjugate Working Dilution (final volume)	Number of strips	Buffer Working Dilution		Conjugate-concentrate	Conjugate Working Dilution (final volume)
1	1,35 ml	+	0,15 ml	1,5 ml	26	35,10 ml	+	3,90 ml	39,0 ml
2	2,70 ml	+	0,30 ml	3,0 ml	27	36,45 ml	+	4,05 ml	40,5 ml
3	4,05 ml	+	0,45 ml	4,5 ml	28	37,80 ml	+	4,20 ml	42,0 ml
4	5,40 ml	+	0,60 ml	6,0 ml	29	39,15 ml	+	4,35 ml	43,5 ml
5	6,75 ml	+	0,75 ml	7,5 ml	30	40,50 ml	+	4,50 ml	45,0 ml
6	8,10 ml	+	0,90 ml	9,0 ml	31	41,85 ml	+	4,65 ml	46,5 ml
7	9,45 ml	+	1,05 ml	10,5 ml	32	43,20 ml	+	4,80 ml	48,0 ml
8	10,80 ml	+	1,20 ml	12,0 ml	33	44,55 ml	+	4,95 ml	49,5 ml
9	12,15 ml	+	1,35 ml	13,5 ml	34	45,90 ml	+	5,10 ml	51,0 ml
10	13,50 ml	+	1,50 ml	15,0 ml	35	47,25 ml	+	5,25 ml	52,5 ml
11	14,85 ml	+	1,65 ml	16,5 ml	36	48,60 ml	+	5,40 ml	54,0 ml
12	16,20 ml	+	1,80 ml	18,0 ml	37	49,95 ml	+	5,55 ml	55,5 ml
13	17,55 ml	+	1,95 ml	19,5 ml	38	51,30 ml	+	5,70 ml	57,0 ml
14	18,90 ml	+	2,10 ml	21,0 ml	39	52,65 ml	+	5,85 ml	58,5 ml
15	20,25 ml	+	2,25 ml	22,5 ml	40	54,00 ml	+	6,00 ml	60,0 ml
16	21,60 ml	+	2,40 ml	24,0 ml	41	55,35 ml	+	6,15 ml	61,5 ml
17	22,95 ml	+	2,55 ml	25,5 ml	42	56,70 ml	+	6,30 ml	63,0 ml
18	24,30 ml	+	2,70 ml	27,0 ml	43	58,05 ml	+	6,45 ml	64,5 ml
19	25,65 ml	+	2,85 ml	28,5 ml	44	59,40 ml	+	6,60 ml	66,0 ml
20	27,00 ml	+	3,00 ml	30,0 ml	45	60,75 ml	+	6,75 ml	67,5 ml
21	28,35 ml	+	3,15 ml	31,5 ml	46	62,10 ml	+	6,90 ml	69,0 ml
22	29,70 ml	+	3,30 ml	33,0 ml	47	63,45 ml	+	7,05 ml	70,5 ml
23	31,05 ml	+	3,45 ml	34,5 ml	48	64,80 ml	+	7,20 ml	72,0 ml
24	32,40 ml	+	3,60 ml	36,0 ml	49	66,15 ml	+	7,35 ml	73,5 ml
25	33,75 ml	+	3,75 ml	37,5 ml	50	67,50 ml	+	7,50 ml	75,0 ml

Test procedure

1.	**Rinse the incubation tray with Buffer Working Dilution and decant liquid**	Mark the trays with water-resistant pen. Rinsing removes dust particles.
2.	**Place one strip per test into each channel**	Place one strip for each control and each patient sample into separate incubation tray channels with forceps. The green line of the strip must face up - antigens are bound to this side.
3.	**Fill each channel with 1.5 ml Buffer Working Dilution and incubate 5 min at room temperature (RT) on a platform rocker**	Visually check to make sure strips are completely wet. Use a platform rocker with a rocking frequency of 40 cycles/min. Avoid spilling of buffer. Do not decant buffer after incubation.
4.	**Add 20 µl of each patient sample or 100 µl of each control**	Pipette controls and samples directly onto the numbered end of the strips while the platform rocker is stopped with numbered end of the strips in the full down position.
5.	**Incubate 30 min at RT on a platform rocker**	Visually check to make sure strips are completely wet. Use a platform rocker with a rocking frequency of 40 cycles/min. Avoid spilling of liquid.
6.	**Decant the liquid**	Remove the remaining liquid by carefully tapping the incubation tray on absorbent paper. **Strips adhere to the incubation tray** when the liquid is decanted.
7.	**Wash 3 times:** **- add 1.5 ml Buffer Working Dilution** **- incubate 5 min by rocking at RT** **- decant the liquid completely**	Wash on the platform rocker. While washing, prepare the Conjugate Working Dilution as described in table 1. Tap the incubation tray on absorbent paper to remove the remaining liquid.
8.	**Add 1.5 ml Conjugate Working Dilution**	Make sure that antigen strips are completely covered with Conjugate Working Dilution.
9.	**Incubate 15 min at RT on a platform rocker**	Visually check to make sure strips are completely wet. Use a platform rocker with a rocking frequency of 40 cycles/min. Avoid spilling of liquid.
10.	**Decant the liquid**	Remove the remaining liquid by carefully tapping the incubation tray on absorbent paper.
11.	**3 times washing as in step 7**	
12.	**Add 1.5 ml distilled water and incubate 1 min at RT on a platform rocker**	Visually check to make sure strips are completely wet. Use a platform rocker with a rocking frequency of 40 cycles/min. Avoid spilling of liquid.
13.	**Decant the liquid**	Remove the remaining liquid by carefully tapping the incubation tray on absorbent paper.
14.	**Add 1.5 ml Chromogen/Substrate Solution**	Visually check to make sure strips are completely wet.
15.	**Incubate at RT on a platform rocker** **Incubation time: 7 to 15 min**	Stop the reaction when the cut off control band is clearly visible. **Caution:** Avoid longer incubation as background staining might occur. **Cut off control band for IgG is located in the strip control section!**
16.	**Stop the reaction by decanting the liquid**	Remove the remaining liquid by carefully tapping the incubation tray on absorbent paper.
17.	**3 times washing with 1.5 ml distilled water**	Wash without incubation time.
18.	**Dry strips for interpretation**	Remove the remaining liquid by carefully tapping the incubation tray on absorbent paper. Place wet strips on unbleached absorbent paper and allow to air dry before band interpretation.

Test principle

The Bordetella pertussis ViraStripe® IgG Testkit enables the detection of Bordetella specific antibodies in human serum.
During the serum incubation step, specific antibodies bind to the immobilised antigens on the strip. During the conjugate incubation step, AP-conjugate binds to this antigen-antibody complex. The alkaline phosphatase reacts with the chromogen/ substrate and stains the antigen-antibody complex on the strip with a purple color.

The washing procedures between serum-, conjugate- and chromogen/substrate incubation remove unbound antibodies and reagents.

Immunoblot with integrated control system:
The green separation line divides the strip into a control section and an analytical section. The control section contains a serum control, three conjugate controls (IgG, IgA, IgM) and a cut off control. The analytical section contains Bordetella species specific antigens.

Interpretation

1. Protocol sheet:
   Record test data on the protocol sheet. Glue or tape the strips onto the protocol sheet. Place the green separation line of the strips exactly onto the separation line printed on the protocol sheet.

2. Validity of the test:
   The test is considered as valid if:
   - the serum control band is clearly visible.
   - one of the three conjugate control bands is clearly visible. If more than one conjugate control band is visible the band with the highest intensity indicates the conjugate being used.
   - the cut off control band is visible.

   Do not assess invalid strips!

3. Assignment of bands:
   The green separation line of the strips indicates position and orientation for the assignment of bands. Assign bands on the patient strips with the band locator printed on the protocol sheet and record results in the intended table.

4. Assessment of bands:
   The use of a cut off control for each test run is recommended (3). **The cut off control band of the Bordetella pertussis ViraStripe® IgG Testkit is located on the strip in the control section.** The intensity of the cut off control band indicates the threshold, as of bands can be assessed.

   Bands are considered as distinct, if their intensity is equal to or higher than the intensity of the cut off control band. Distinct bands are marked with an "**X**" on the protocol sheet.

   Bands are considered as weak, if their intensity is lower than the intensity of the cut off control band. Weak bands are marked with an "**(X)**" on the protocol sheet.

   Bands are not assessed if they are not visible on the strip.

   Record bands and results on the protocol sheet.

5. Evaluation:
   Bands FHA, PT-100 and PT must be assessed on the Bordetella pertussis ViraStripe® IgG. FHA may also react positive while contact with Bordetella species. Bordetella pertussis toxin is applied in two different bands: PT-100 and PT.
   A distinct PT band indicated the presence of IgG antibodies against Bordetella pertussis toxin. A distinct PT-100 band indicates the presences of a high antibody concentration (at least 100 IU/ml) against Bordetella pertussis toxin.

Nomenclature and description of Bordetella pertussis ViraStripe® IgG bands

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 (4). Antibodies to FHA are developed after vaccination and after infection with Bordetella pertussis or Bordetella parapertussis.
PT-100 (28 kD) Pertussis Toxin	Highly specific for Bordetella pertussis. The PT-100 band is calibrated with International WHO standards and when present in cut off intensity correlates with a value around 100 IU/ml (5,6,7). Following RKI guidelines, a once clearly increased IgG value presents supportive evidence for a recent contact with Bordetella pertussis (2). This value corresponds with a distinct PT-100 band.
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 (4). Antibodies against PT are developed after vaccination and after infection with Bordetella pertussis but not after infection with Bordetella parapertussis. The PT band is calibrated with International WHO standards and when present in cut off intensity correlates with a value around 8 IU/ml (5,8).

Interpretation of results

Bands have to be considered as symptoms of the disease. For a final clinical diagnosis all results from this and other tests must be correlated with clinical history, epidemiological data and other data available to the attending physician. The bands **PT and PT-100 (28 kD)** are characterized as **highly specific** for Bordetella pertussis. The band **FHA (220 kD)** is characterized as **highly specific** for Bordetella species**.**

Identified bands	Assessment / Result	Interpretation
Distinct FHA band	**Positive**	IgG antibodies against **Bordetella species** detectable.
Weak FHA band	**Equivocal**	Low antibody titers against **Bordetella species** detectable. In case of a clinical suspected Bordetella species infection, check for anti-PT and anti-FHA IgA antibodies and/or check a second patient sample after 2-3 weeks.
No FHA band	**Negative**	No specific IgG antibodies against **Bordetella species** detectable.
Distinct PT band and **Distinct PT-100** band	**Positive**	IgG antibodies against **Bordetella pertussis** detectable. Evidence for a recent contact with Bordetella pertussis (1). This band scheme corresponds to an anti-PT antibody titer of at least 100 IU/ml. Following RKI guidelines this correlates with a once clearly increased IgG value (2).
Distinct PT band and **No** or **weak PT-100** band	**Positive**	IgG antibodies against **Bordetella pertussis** detectable. Suspicion of recent contact with Bordetella pertussis. To clarify, check for anti-PT and anti-FHA IgA antibodies and/or check a second patient sample after 2-3 weeks. This band scheme corresponds to an anti-PT antibody titer of at least 8 IU/ml.
No or **weak PT** band and **No PT-100** band	**Negative**	No specific IgG antibodies against **Bordetella pertussis** detectable.

Table 2: Bordetella pertussis ViraStripe® IgG and IgA PT band results and consequential diagnostic interpretation

IgG PT-100	IgG PT	IgA PT	Assessment for IgG and IgA together	Interpretation of IgG und IgA PT band results
Ø	Ø	Ø	**Negative**	No specific antibodies against Bordetella pertussis detectable. No evidence for an infection and no vaccine titer present. In case of a clinical suspected Bordetella pertussis infection, check a second patient sample after 2-3 weeks.
Ø	+	Ø	**Positive**	IgG antibodies against Bordetella pertussis detectable. A vaccine titer and/or a residual antibody titer after infection are likely.
+	+	Ø	**Positive**	IgG antibodies against Bordetella pertussis detectable. An infection is likely. A vaccine titer may be supposable if a vaccination has occured recently.
Ø	Ø	+	**Positive**	IgA antibodies against Bordetella pertussis detectable. An early stage infection is likely. IgA antibodies are rarely seen after vaccination (16,17).
Ø	+	+	**Positive**	IgG and IgA antibodies against Bordetella pertussis detectable. An infection is likely. IgA antibodies are rarely seen after vaccination (16,17).
+	+	+	**Positive**	IgG and IgA antibodies against Bordetella pertussis detectable. An infection is likely. IgA antibodies are rarely seen after vaccination (16,17).

Performance characteristics of the Bordetella pertussis ViraStripe® IgG Testkit

Sensitivity:

Analytic sensitivity of the Bordetella pertussis ViraStripe® IgG Testkit has been identified by analysing WHO standards. These standards contain a defined amount of anti-PT and anti FHA antibodies (IgG and IgA), measured in International Units per Millilitre (IU/ml) (5).

PT band: Distinct band starting around 8 IU/ml, based on anti-PT IgG antibodies.
PT-100 band: Distinct band starting around 100 IU/ml, based on anti-PT IgG antibodies.
FHA band: Distinct band starting around 20 IU/ml, weak band starting around 8 IU/ml, both based on anti-FHA IgG antibodies.

Specificity:

Specificity of the Bordetella pertussis ViraStripe® IgG Testkit has been identified by analysing 146 unselected blood donors.

Table 3: Blood donor analysis with Bordetella pertussis ViraStripe® IgG Testkit

Bordetella pertussis ViraStripe® IgG	Positive with PT band, % (n)	Positive with PT-100 band, % (n)	Positive/equivocal with FHA band, % (n)
Blood donors **(n= 146)**	**48% (70)**	**3,4% (5)**	**84% (123)**

Scientific studies demonstrate the presence of anti-PT IgG antibodies in blood donor sera in 46% of all cases, showing at least the "minimal level of quantitation" (8 IU/ml) (8). This level correlates to a **distinct** Bordetella pertussis ViraStripe® IgG **PT band**. Analysis of 146 unselected blood donor sera with the Bordetella pertussis ViraStripe® IgG Testkit shows in 48% of all cases a positive result with the PT band (see table 3).

A further scientific investigation with 5366 blood donor sera shows that anti-PT IgG antibody titer over 94 UI/ml occurs in 2,89% of all cases, indicating a recent contact with Bordetella pertussis (7). This level correlates to a **distinct** Bordetella pertussis ViraStripe® IgG **PT-100 band**. Analysis of 146 unselected blood donor sera with the Bordetella pertussis ViraStripe® IgG Testkit shows in 3,4% of all cases a positive result with the PT-100 band (see table 3).

Scientific studies demonstrate the presence of anti-FHA IgG antibodies in blood donor sera in 86% of all cases, showing at least the "minimal level of quantitation" (8 IU/ml) (8). This level correlates to a **distinct or weak** Bordetella pertussis ViraStripe® IgG **FHA band**. Analysis of 146 unselected blood donor sera with the Bordetella pertussis ViraStripe® IgG Testkit shows in 84% of all cases a positive or equivocal result with the FHA band (see table 3).

Diagnostic significance of antibodies against Bordetella species

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 (9). IgG antibodies can persist more than 10 years, but at least 6 months after beginning of the disease (9,10). 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 (11). Infants can get maternal IgG antibodies diaplacental (4, 12).
As proof for a recent infection with Bordetella pertussis, the Robert Koch Institute (RKI) recommends with serological testing methods a "once clearly increased value" for IgG antibodies to Bordetella pertussis, if vaccination has occurred more than 36 months ago (2).
This value correlates at least to an anti-PT antibody titer of 100 UI/ml, corresponding to a distinct PT-100 band and presenting supportive evidence for a recent contact with Bordetella pertussis (1,6,7).

2. IgM antibodies usually appear 8-15 days after beginning of the disease (9). They reach their highest concentration approx. 8-10 weeks after beginning of the disease (11). 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 raised after vaccination (13). In singular cases IgM antibodies may appear only weak, delayed or not at all in infants and adults (10).

3. IgA antibodies are nearly exclusively detectable after natural infection and only in very rare cases after vaccination (16,17). IgA antibodies reach their highest concentration approx. 8-10 weeks after beginning of the disease (11). IgA antibodies are generally not longer detectable than 6 months after infection (9). 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 (13).
There are indications for persisting of Bordetella pertussis specific antibodies in the population caused by subclinical infections (13).

4. Detection of antibodies against Pertussis Toxin (PT) is specific for Bordetella pertussis (4,9,12,14).

5. Drugs and antibody therapy can result in unspecific antibody responses (15).

Warnings and precautions

1. All human serum components have been tested and found to be negative for HCV-, HIV1- and HIV2-antibodies and Hbs-antigens. Nevertheless all human kit components and also the patient samples should be considered as potentially infectious and carefully handled accordingly.
2. Do not pipette by mouth.
3. Wear disposable gloves and safety glasses while working.
4. Do not eat, drink or smoke in the working area.
5. Conjugates contain <0.1% sodium azide, controls contain <0.07% thimerosal and diluent/wash buffer contains 0.02% thimerosal as preserving agent. Caution: Injurious to health. In case of contact with skin or eyes, wash with large quantities of water. Poisonous when swallowed! (pay attention to material safety data sheets).
6. The chromogen/substrate solution contains BCIP and NBT. Avoid contact with skin and eyes. In case of contact with skin and eyes wash with large quantities of water.
7. Specimens and all potentially contaminated materials have to be decontaminated using validated laboratory techniques, e.g. by autoclaving 15 min 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.
8. In vitro diagnostics must not be used beyond expiration date as reliable results may not be possible.
9. Follow the Instruction for Use carefully to ensure reliable results.
10. Efficient washing after each incubation step is essential for consistent results; insufficient washing may lead to false positive results.
11. Please refer to material safety data sheets for detailed information on potential risks, accidental release measures, first aid guidelines, handling and storage recommendations, personal protective equipment and recommendations for disposal.
12. While working with potential infectious/hazardous materials, all national and international rules, regulations, guidelines and laws must be observed. This also applies for storage and disposal of used chemicals and reagents.

Specimen handling

1. The **Bordetella pertussis ViraStripe® IgG Testkit** may be used with human serum.
2. Only clear, non-hemolised, non-microbially contaminated specimens must be used.
3. Using icteric, lipemic, hemolytic and/or heat-treated serum may lead to false positive 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. Allow specimens to reach room temperature before testing. Mix specimens carefully after thawing. Precipitates in specimens can be removed by centrifugation.
6. Avoid multiple freeze and thaw cycles.

Literature

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