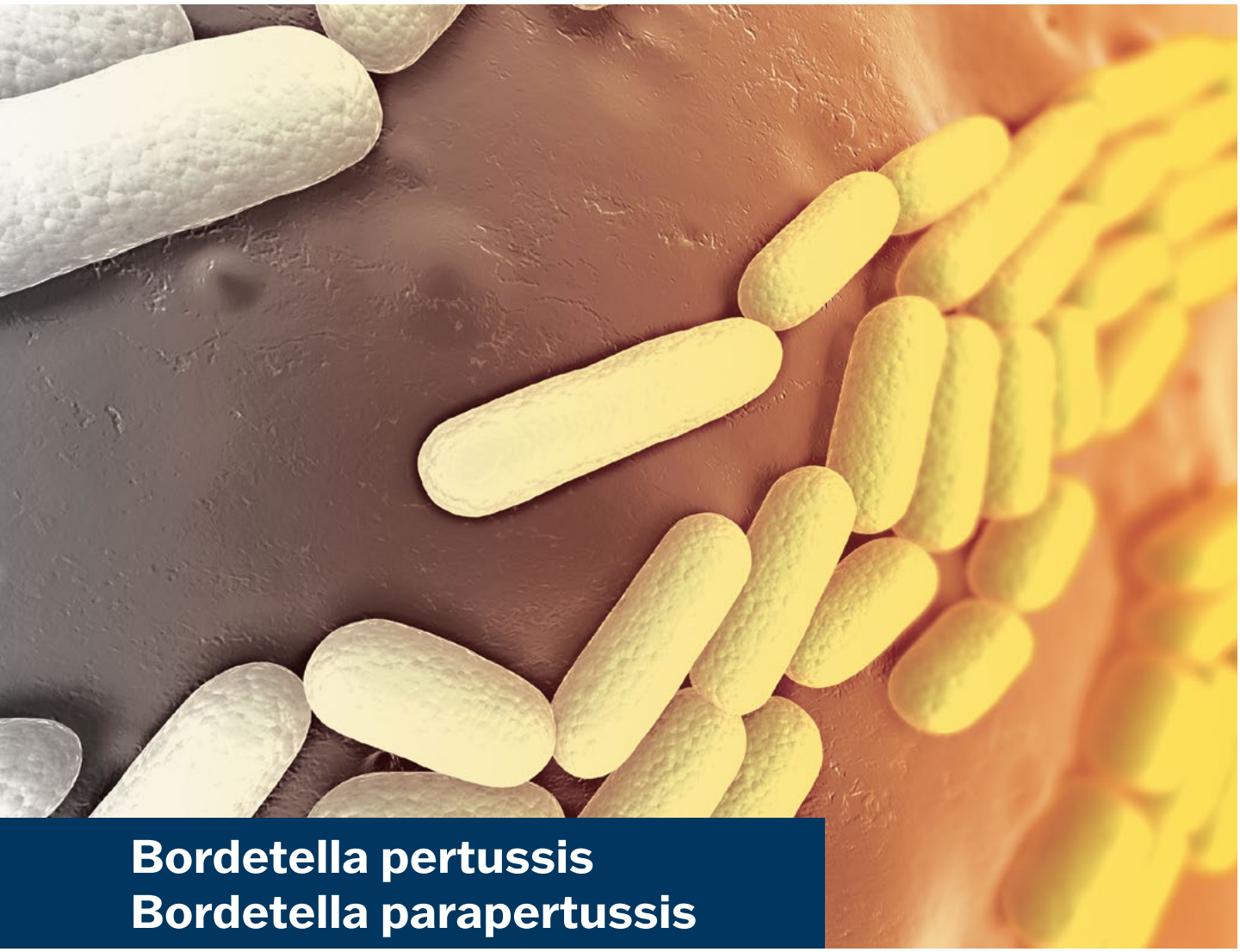


Bordetella pertussis
Bordetella parapertussis

Enzyme immunoassays for the diagnostics of pertussis and parapertussis

ELISA, **IMMUNOBLOT**, and **MICROBLOT-ARRAY** kits are optimized and validated for detection of IgA, IgG, and IgM antibodies in human serum and plasma

Components for agglutination are optimized and validated for detection of all immunoglobulin classes in human serum

CE IVD

Diagnostic kits are intended for professional use in the laboratory.

BG TestLine®

Introduction

Bordetella pertussis is considered to be the main cause of whooping cough. Before a vaccination campaign was launched, the disease had been one of the most serious diseases of infants and children. *B. pertussis* causes the severe form of the disease which lasts 6–8 weeks and has following stages:

Incubation 6–20 days.

Catarrhal (1–2 weeks) – symptoms correspond to common cold symptoms. During this stage dry irritating cough becomes more severe and develops into coughing fits.

Paroxysmal (2–6 weeks) – typical symptom of the disease – fits of violent whooping cough. Number and severity of the fits are increasing; the fits are often accompanied by vomiting.

Convalescent (1–3 weeks) – characterised by decrease in frequency of fits and milder cough.

The common symptoms of pertussis are a paroxysmal cough, inspiratory whoop, and fainting and/or vomiting after coughing. The cough from pertussis has been documented to cause subconjunctival hemorrhages, rib fractures, urinary incontinence, hernias, post-cough fainting, and vertebral artery dissection.

Whooping cough does not induce lifelong immunity. Antibodies against pertussis toxin, filamentous hemagglutinin and fimbrial antigen can be detected in serum.

B. parapertussis causes milder forms of the disease. This is due to the fact that the bacteria do not produce the pertussis toxin. The infection of B. parapertussis can be the main cause of prolonged bronchitis.

Postvaccination immunity and immunity following *B. pertussis* infection do not protect from the disease caused by *B. parapertussis*.

Diagnosis of Infection

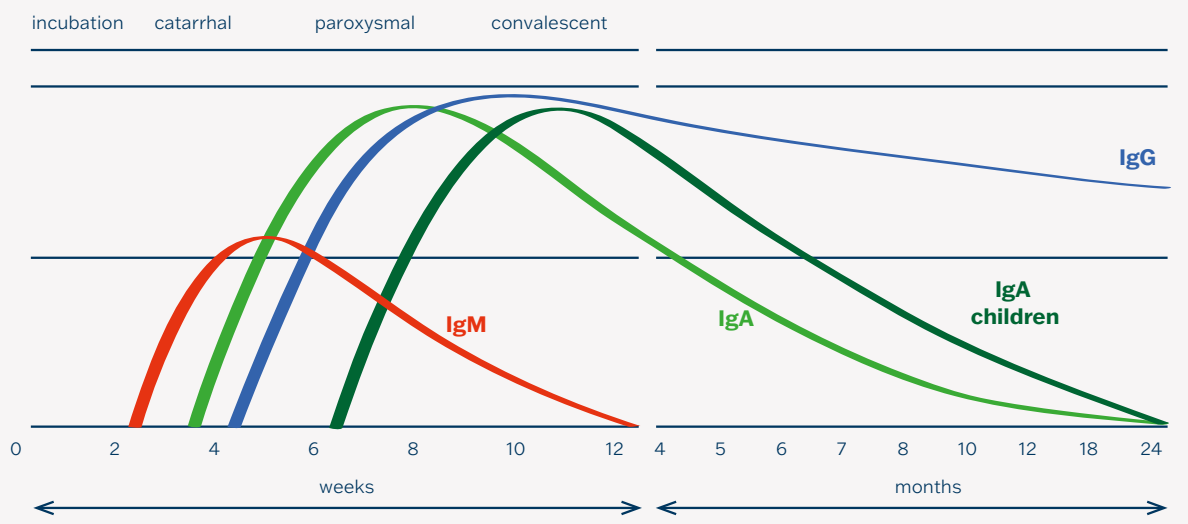
Clinical picture of the disease and epidemiologic anamnesis are supplemented by laboratory tests (direct detection by cultivation or PCR, and specific antibodies determination).

Serological examination is based on determination of IgA, IgG and IgM specific antibodies. IgM antibodies are detected first; they have short half-life and endure for 2–3 months. IgA antibodies can be determined after 1–2 weeks and may persist for 6–24 months depending on age. IgG antibodies are found first after 2–3 weeks after the onset of the disease and reach their maximum after 6–8 weeks. They can be detected till adulthood and may persist for several years.

In children, IgA antibodies are produced more slowly – they reach detectable level 6–7 weeks after infection in infants. The detection of specific IgM antibodies is suitable for diagnosis of the acute disease in younger children while specific IgA antibodies show better diagnostic potential in older children.

Serological findings should be interpreted in the context of clinical picture, vaccination and epidemiologic data, and available results of other laboratory tests. Examination should be repeated in case of ambiguous results after 2–3 weeks according to patient's clinical status.

Antibody Response



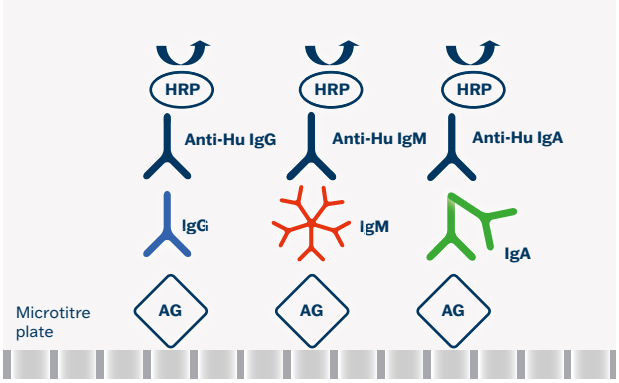
Interpretation of *B. pertussis*/*B. parapertussis* results

IgG	IgA	IgM	Interpretation
+	+	+	Presence of IgA, IgG or IgM antibodies – recent or current natural infection
+	+	–	
+	–	+	Presence of IgG and IgM antibodies in the absence of IgA antibodies – state after recent vaccination (*B. pertussis*) or an early infection stage without IgA antibodies production
–	+	+	Presence of IgA antibodies or parallel presence of IgM antibodies – early infection stage
–	+	–	
–	–	+	Presence only of IgM antibodies – early infection stage
+	–	–	Presence only of IgG antibodies – recent infection or postvaccination state (*B. pertussis*)
–	–	–	No presence of anti *B. pertussis* or anti *B. parapertussis* antibodies – in the case of a suspected

ELISA

Test Principle

The assays are based on a sandwich type of ELISA method.



Summary Protocol

Step		Test steps
	1.	Dilution of samples – serum/plasma 1:101 (10 µl + 1 ml)
	2.	Pipette Controls and diluted samples 100 µl – Including blank
	3.	Incubate 30 min. at 37 °C
	4.	Aspirate and wash the wells 5 times
	5.	Add Conjugate 100 µl – Including blank
	6.	Incubate 30 min. at 37 °C
	7.	Aspirate and wash the wells 5 times
	8.	Add 100 µl Substrate (TMB-Complete) – Including blank
	9.	Incubate 15 min. at 37 °C
	10.	Add 100 µl Stopping solution – Including blank
	11.	Read colour intensity at 450 nm

Antigens

EIA *Bordetella pertussis*

Highly purified Bordetella pertussis toxin

EIA *Bordetella parapertussis*

Mixture of specific antigens for Bordetella parapertussis

Clinical Application

- Screening test for the detection of specific IgA, IgG and IgM antibodies in human serum or plasma
- Detection of postinfection and postvaccination antibodies (*B. pertussis*)
- Disease stage diagnosis

User Comfort

- Ready-to-use components
- Colour-coded, interchangeable components
- Breakable colour-coded microplate strips
- Calibrators and Controls
- Quantitative evaluation of results (U/ml)
  - *B. pertussis*
- Semiquantitative evaluation of results (IP)
  - *B. parapertussis*
- Standardization according to WHO International Standard Pertussis Antiserum 06/140 and 06/142
- High reproducibility and dynamics of antibody response
- Identical assay procedure, ready for automation
- Short total assay time

Test Characteristics

ELISA	Diagnostic Sensitivity	Diagnostic Specificity
EIA Bordetella pertussis Toxin IgA	95.8%	99.9%
EIA Bordetella pertussis Toxin IgG	97.1%	99.9%
EIA Bordetella pertussis Toxin IgM	90.5%	92.0%
EIA Bordetella parapertussis IgA	93.3%	99.9%
EIA Bordetella parapertussis IgG	96.7%	99.9%
EIA Bordetella parapertussis IgM	68.8%	99.9%

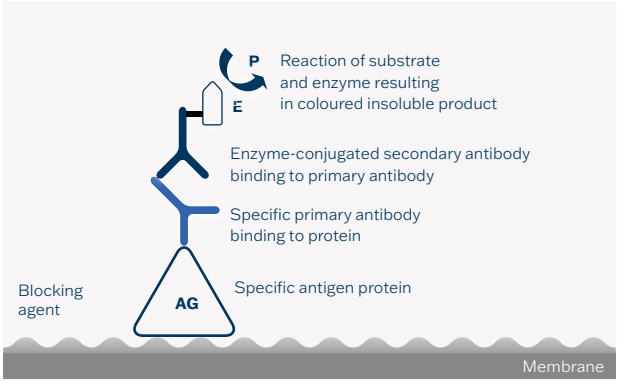
EIA

SmartEIA

IMMUNOBLOT

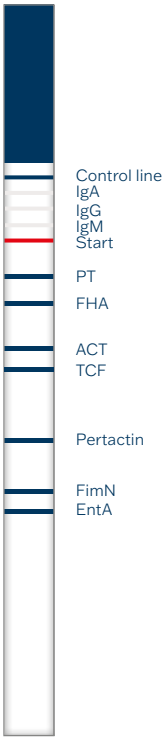
Test Principle

Recombinant antigens are transferred to a nitrocellulose membrane using a micro-dispensing method.



Antigens

BLOT-LINE
Bordetella



B. pertussis

PT – Pertussis toxin (45 kDa) – basic virulence factor, specific only for *B. pertussis*; the most important pertussis antigen

FHA – *B. pertussis* filamentous hemagglutinin – adhesive protein, important immunogen; selected part of the sequence with high specificity

ACT – Adenylate cyclase toxin (CyaA) – important virulence factor of *B. pertussis*; antiphagocytic factor during infection

TCF – Tracheal colonization factor – protein produced only by *B. pertussis* strain, not by *B. parapertussis*; protein adhesin, that binds to ciliated epithelial cells of respiratory tract

B. parapertussis

Pertactin – Outer membrane protein (75 kDa) of virulent B. parapertussis strains

FimN Fimbriae N – protein adhesin; it is not produced by *B. pertussis*

EntA Entericidin A – membrane lipoprotein

Summary Protocol

Step		Test steps
	1.	Pipette Universal solution 2.5 ml
	2.	Strips soaking 10 min. at room temperature – Shaker
	3.	Aspirate
	4.	Dilute samples – serum/plasma 1:51 (30 µl + 1.5 ml)
	5.	Pipette Controls and diluted samples 1.5 ml
	6.	Incubate 30 min. at room temperature – Shaker
	7.	Aspirate samples and wash strips with 1.5 ml of Universal solution 3-times for 5 min. – Shaker
	8.	Pipette Conjugate 1.5 ml
	9.	Incubate 30 min. at room temperature – Shaker
	10.	Aspirate Conjugate and wash strips with 1.5 ml of Universal solution 3-times for 5 min. – Shaker
	11.	Pipette Substrate solution (BCIP/NBT) 1.5 ml
	12.	Incubate 15 min. at room temperature – Shaker
	13.	Aspirate Substrate solution and wash strips with 2 ml of distilled water 2-times for 5 min. – Shaker
	14.	Sticking and evaluation of strips

Clinical Application

- Differentiation of postinfection and postvaccination antibodies
- Proof of acute infection
- Differential diagnostics of *B. pertussis* and *B. parapertussis*
- Detailed determination of the presence of anti-Bordetella specific antibodies
- Confirmation of ELISA and/or agglutination tests

User Comfort

- Ready-to-use components
- Colour-coded strips
- Positive and Negative controls
- Interchangeable components
- Possibility of automation
- Easy assay procedure

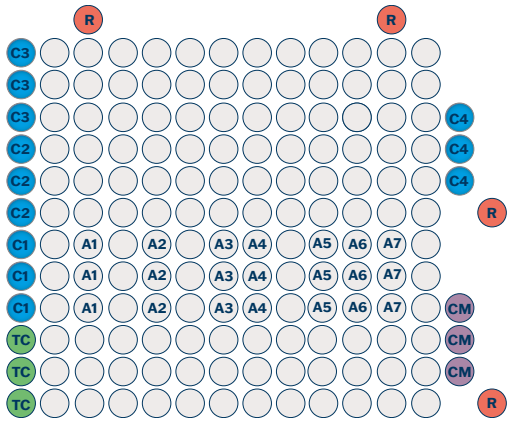
Test Characteristics

Pathogen	Diagnostic Sensitivity	Diagnostic Specificity
Bordetella pertussis IgA	95.5%	95.6%
Bordetella pertussis IgG	99.0%	95.6%
Bordetella parapertussis IgA	99.0%	87.0%
Bordetella parapertussis IgG	88.9%	96.4%

IMMUNOBLOT

MICROBLOT-ARRAY

Distribution of Antigens and Control Spots



Description of antigens

A1 – PT
A2 – FHA
A3 – ACT
A4 – TCF
A5 – Pertactin
A6 – FimN
A7 – EntA

Description of control spots

R – Reference
TC – Test control
CA – Conjugate control IgA
CG – Conjugate control IgG
CM – Conjugate control IgM
C1 – Calibration 1
C2 – Calibration 2
C3 – Calibration 3
C4 – Calibration 4

Protocol Summary

Step		Test steps
	1.	Pipette Universal solution 150 µl
	2.	Strips soaking 10 min. at room temperature
	3.	Aspirate
	4.	Dilute samples – serum/plasma 1:51 (10 µl + 500 µl)
	5.	Pipette Controls and diluted samples 100 µl
	6.	Incubate 30 min. at room temperature
	7.	Quick wash using the Universal Solution*
	8.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	9.	Pipette Conjugate 100 µl
	10.	Incubate 30 min. at room temperature
	11.	Quick wash using the Universal Solution*
	12.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	13.	Pipette Substrate solution (BCIP/NBT) 100 µl
	14.	Incubate 15 min. at room temperature
	15.	Quick wash using the distilled water*
	16.	Aspirate Substrate solution and wash strips with 200 µl of distilled water 2-times for 5 min.
	17.	Dry and evaluate strips

* In case of using the washer fill the wells up to the rim and aspirate immediately after filling the last well.

User Comfort

- Low sample consumption
- Antigens spotted in triplicate – minimizing statistical variation
- Possibility of automatic assay processing and results evaluation
- Parallel testing of multiple markers simultaneously
- High sensitivity and specificity

Microblot-Array

Test Characteristics

Pathogen	Diagnostic Sensitivity	Diagnostic Specificity
Bordetella pertussis IgA	95.4%	100.0%
Bordetella parapertussis IgA	96.9%	100.0%
Bordetella pertusis IgG	97.6%	100.0%
Bordetella parapertussis IgG	97.1%	100.0%
Bordetella pertussis IgM	95.4%	100.0%
Bordetella parapertussis IgM	95.8%	100.0%

Ordering Information

ELISA

Cat. No.	Product	No. of Wells
BpAT96	EIA Bordetella pertussis Toxin IgA	96
BpGT96	EIA Bordetella pertussis Toxin IgG	96
BpMT96	EIA Bordetella pertussis Toxin IgM	96
BppA96	EIA Bordetella parapertussis IgA	96
BppG96	EIA Bordetella parapertussis IgG	96
BppM96	EIA Bordetella parapertussis IgM	96
SK-BpAT96	SmartEIA Bordetella pertussis Toxin IgA	96
SK-BpGT96	SmartEIA Bordetella pertussis Toxin IgG	96
SK-BpMT96	SmartEIA Bordetella pertussis Toxin IgM	96
SK-BppA96	SmartEIA Bordetella parapertussis IgA	96
SK-BppG96	SmartEIA Bordetella parapertussis IgG	96
SK-BppM96	SmartEIA Bordetella parapertussis IgM	96

SmartEIA kits are designed for automated processing using the Agility® analyser

IMMUNOBLOT

Cat. No.	Product	No. of Tests
BpAL20	BLOT-LINE Bordetella IgA	20
BpGL20	BLOT-LINE Bordetella IgG	20
BpML20	BLOT-LINE Bordetella IgM	20
BD-BpAL24	BlueBLOT-LINE Bordetella IgA	24
BD-BpGL24	BlueBLOT-LINE Bordetella IgG	24
SwIm03	Immunoblot Software	1 pc

The BlueBLOT-LINE kits are designed for automatic processing using BlueDiver® analyser

Ordering Information

MICROBLOT-ARRAY

Cat. No.	Product	No. of Wells
BpAMA48	Microblot-Array Bordetella IgA	48
BpGMA48	Microblot-Array Bordetella IgG	48
BpMMA48	Microblot-Array Bordetella IgM	48

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CERTIFIED
ISO 9001 · ISO 13485

Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.

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