Instructions for use

For *in vitro* diagnostic use only

Bordetella pertussis IgA EIA

A solid-phase enzyme immunoassay for the detection of IgA antibodies to *Bordetella pertussis* in human serum or plasma.

Product no. 61 11 510 (Microstrips®, 96 wells)

CE

Date of issue: December 30, 2005

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INTENDED USE

Ani Labsystems' Bordetella pertussis IgA EIA kit is intended for the detection of IgA-class antibodies to *Bordetella pertussis* in human serum and plasma, and is used as an aid for the diagnosis of *Bordetella pertussis* infection.

The test is recommended to be run and interpreted in parallel with Ani Labsystems' Bordetella pertussis IgG and IgM EIAs kits.

INTRODUCTION

Pertussis is an acute, highly communicable infection of the respiratory tract caused by *Bordetella pertussis* bacteria. The disease is transmitted by respiratory droplets from infected individuals. The incubation period is 6-20 days. The infectivity is greatest early in the disease and decreases until the pathogen disappears from the nasopharynx. (1 – 3)

Bordetella pertussis interferes with ciliary activity and this is responsible for obstruction of airways, because the transport and clearance of mucus is diminished. In addition to directly injuring ciliated epithelial cells *Bordetella pertussis* excretes different toxins possibly involved in the pathogenesis of whooping cough (4).

The characteristic features of pertussis are paroxymal coughing with an inspiratory whoop and/or lymphocytosis (4). In young infants pertussis is a serious disease that can be complicated by pneumonia, convulsions, brain damage, atelectasis, interstitial and subcutaneous emphysema and pneumotorax. In older children and adults the disease is usually mild characterized by a cough lasting 1-3 months. The patient with a typical pertussis may transmit the disease to infants (5).

The definite method of diagnosing whooping cough is by isolating *Bordetella pertussis* in clinical specimens. There are, however, many difficulties in culturing, and most studies report recovery rates < 60 % (6). Isolation is most effective in young infants, in later life the isolation rate decreases strikingly but because of the long generation time of *Bordetella pertussis* the diagnosis can be obtained only after 3 to 7 days (7). Direct immunofluorescent staining of nasopharyngeal secretions has been used for diagnosis, but this test lacks specificity. Antibody response to *Bordetella pertussis* has been studied extensively, and several serologic studies have been performed with varying results (8-10).

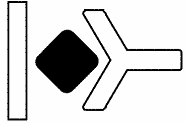
For the primary diagnosis of the whooping cough in the acute phase, determination of specific IgA- and IgM-class antibodies is recommended. In the prolonged course of the disease, rise in specific IgG-class antibodies gives valuable information for diagnosis.

The enzyme-linked immunosorbent assay (ELISA) was first described by Engvall and Perlman (11), and it has been used for measuring antibodies to a wide variety of antigens. Ani Labsystems' Bordetella pertussis IgA EIA Kit is a solid-phase enzyme immunoassay of the ELISA type.

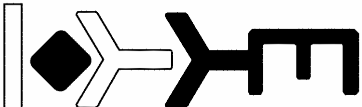
PRINCIPLE OF THE TEST

The principle of the Ani Labsystems' Bordetella pertussis IgA EIA kit is based on an indirect solid-phase enzyme immunoassay with horseradish peroxidase as a marker enzyme. The assay proceeds according to the following reactions.

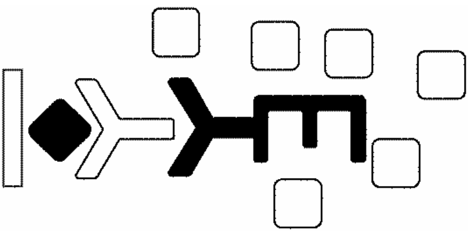
1. *Bordetella pertussis* IgA antibodies from the patient sample ( ) bind to *Bordetella pertussis* antigen ( ) attached to the polystyrene surface ( ) of the Microstrip® wells.



2. Residual patient sample is removed by washing and horseradish peroxidase conjugated anti-human IgA (sheep) ( ) is added.



3. Unbound conjugate is washed off and a colourless enzyme substrate (H_2O_2) containing the chromogen (TMB*) is added. The enzyme reaction with the chromogen results in a coloured end product.



4. The colour formation reaction is terminated by adding acid (H_2SO_4). The colour intensity is directly proportional to the concentration of *Bordetella pertussis* antibodies in a patient sample.

* Tetramethylbenzidine, a non-mutagenic chromogen for horseradish peroxidase.

KIT CONTENTS

Note: Prewarm all reagents and Microstrips® to +20°C - +25°C and incubator to +37°C before use.

– Wear disposable gloves while handling specimens and kit reagents. Wash hands carefully after handling of samples.
– Reagents are stored between +2°C and +8°C.
– The expiration date is printed on each component label and on the package.
– Avoid unnecessary exposure to light. This is merely a precaution. The light sensitive reagents are the conjugate and the TMB-substrate solution, which are packaged in non-transparent plastic vials for protection.
– Once opened the components must be resealed tightly.

1 MICROSTRIPS®, 12 x 8 wells
Coated Microstrips®.

2 SAMPLE DILUENT, 100 ml
Phosphate buffered saline with proprietary additives, a blue colouring reagent, and 0.05 % Bronidox® as preservative.

3a NEGATIVE CONTROL , 1 ml
Diluted human serum with 0.05 % Bronidox® as a preservative and a red colouring reagent.

3b POSITIVE CONTROL , 1 ml
Diluted human serum with 0.05 % Bronidox® as a preservative and a red colouring reagent.

4 CONJUGATE, 30 ml
Buffered salt solution with proprietary additives, a red colouring reagent, horseradish peroxidase conjugated anti-human IgA (sheep) with 0.1% N-Methylisothiazolone as preservative.

5 TMB-SUBSTRATE SOLUTION, ready to use ,18 ml
Citrate buffered solution of 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide with proprietary additives and 0.01% Kathon CG as preservative.

6 STOPPING SOLUTION, 25 ml
0.45 M H_2SO_4

7 WASHING SOLUTION, 100 ml
Concentrated citrate buffered saline, with proprietary additives, and 0.05 % Bronidox® as preservative.

INCUBATION COVERS, 2 pcs

REAGENT BASINS, 6 pcs

REAGENT PREPARATION

Table 1.

Reagent	Preparation	Stability of opened/diluted reagents (+2°C to +8°C)
1 Coated Microstrips®	Ready for use	6 months *)
2 Sample diluent	Ready for use	6 months *)
3 Controls	Ready for use	6 months *)
4 Conjugate	Ready for use	6 months *)
5 TMB- Substrate solution	Ready for use	6 months *) Discard unused reagent from the reaction basin. A deep blue color present in the substrate indicates that the solution has been contaminated and must be discarded.
6 Stopping solution	Ready for use	6 months *)
7 Washing solution concentrate (10x)	Dilute the concentrate (vial 7) 1+9 (1:10) with distilled water	6 months *)
Washing solution		1 month at +4°C or 1 week at room temperature

*) Once the Microstrip® foil-package is opened it should be resealed tightly with the desiccant: fold the opened end a few times and seal air-tightly with tape over the whole length of the opening. Store at +2°C to +8°C. The stability of the opened reagents is the maximum only if they are stored properly at +2°C to +8°C. However,

high environmental temperature and contamination may decrease the stability.

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water, preferably sterile.
- Graduated cylinders for reagent dilution.
- Vials to store the diluted reagents.
- Precision pipettes (one channel eg. 0.5-10 µl, 5-50 µl, 20-200µl, 100-1000 µl ranges and multi-channel 50-300 µl)
- Paper towels or absorbent paper.
- Timer, 60 min range.
- Microplate incubator
- Microplate photometer, 450 nm
- Microplate washer
- Sodium hypochlorite solution, free available chlorine 50-500 mg/l.
- Disposable gloves.

PRECAUTIONS

For *in vitro* diagnostic use only.

Warning - POTENTIAL BIOHAZARDOUS MATERIAL:

Each donor unit used in the preparation of the controls sera in the kit has been tested for the presence of the antibodies to HIV (Human Immunodeficiency Virus) and HCV (Hepatitis C Virus) as well as Hepatitis B surface antigen (HBsAg) and found to be non-reactive. Because no test method can offer complete assurance that HIV, hepatitis B virus, HCV, or other infectious agents are absent, these calibrators and controls as well as specimens should be handled at the Biosafety level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes for Health Manual, “Biosafety in Microbiological and Biomedical Laboratories,” 1999.

Discard all materials and specimens as if capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C. Liquid wastes not containing acid and neutralised waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-500 mg/l free available chlorine. Allow 30 minutes for decontamination to be completed.

Note: Liquid waste containing acid must be neutralised with a proportional amount of base prior to the addition of sodium hypochlorite.

Spills should be wiped off thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe off spills should be added to biohazardous waste matter for proper disposal.

Wear disposable gloves while handling specimens and kit reagents. Afterwards wash hands carefully. Never pipette by mouth.

Stopping solution (vial 6) contains 0.45 M sulphuric acid, avoid contact with skin and eyes.

Avoid unnecessary exposure to light. The light sensitive reagents are the conjugate and the TMB-substrate solution, which are packaged in non-transparent plastic vials for protection.

All reagents and Microstrips® must be warmed up to +20°C - +25°C before use.

Do not use reagents after the expiration date printed on the label.

Do not mix or interchange reagents from different lots. Do not interchange vial caps.

When removing aliquots from the reagent vials, use aseptic technique to avoid contamination, or erroneous results may occur. Use a new pipette tip for each sample. Do not touch the wells or splash reagents while pipetting. Reusable glassware must be disinfected, washed out and rinsed free of detergents.

Accurate and precise pipetting, as well as following the exact time and temperature requirements, is essential.

Once the assay has been started, all subsequent steps should be performed without interruption. Do not let the wells dry once the assay has been started.

Do not reuse a Microstrip® even if some wells were not used.

SPECIMEN COLLECTION AND HANDLING

Serum and plasma samples should be refrigerated (+4°C) after collection or, if the test cannot be performed within 48 hours, frozen (-20°C or -70°C, which is preferred). **Samples should not be repeatedly frozen and thawed.**

Do not use sodium azide as preservative because it inactivates horseradish peroxidase.

Heat inactivation of serum or plasma (+56°C, 30 min.) may cause non-specific results.

Microbially contaminated, grossly hemolyzed or hyperlipemic serum and plasma may give erroneous results.

Long storage of serum (frozen over one year) may cause the formation of lipid aggregates. These aggregates may cause a non-specific result.

TEST PROCEDURE

OUTLINE OF PROCEDURE

STEP 1
Mix in a tube **500 µl** sample diluent and **5 µl** specimens (dilution 1:101)

Pipette **100 µl** of sample diluent in well A1 and B1 (blank).

Pipette in duplicates **100 µl** of each control (3a and 3b) in well C1 to F1

Pipette by a multichannel pipette **50 µl** of the sample diluent to each well (except A1 to F1)

Pipette **50 µl** of diluted specimen into the Microstrip® wells

Cover the plate and incubate **1h at +37°C**

Wash **5 x 300 - 400 µl/well**

STEP II
Add **100 µl** conjugate solution.

Cover the plate and incubate **1h at +37°**

Wash **5 x 300 - 400 µl/well**

STEP III
Add **100 µl** TMB-substrate solution

Incubate **30 min at RT (+20°C - +25°C) in dark**

STEP IV

Add **100 µl** stopping solution

Measure at **450 nm**

PRELIMINARY PREPARATIONS

- Wear disposable gloves throughout the procedure!
- **Bring the reagents and Microstrips® to room temperature (+20°C to +25°C) before starting the assay.**
- Prewarm the incubator to +37°C.
- Do not let the wells dry once the assay has been started.

SPECIMEN DILUTION: Dilute the specimen 1:101 in sample diluent (5 µl serum or plasma sample and 500 µl of sample diluent). Mix well.

NOTE: Do not dilute the controls!

NOTE: All *Mycoplasma pneumoniae, Chlamydia pneumoniae* and *Bordetella pertussis* antibodies (IgG, IgA and IgM) can be determined from the same prediluted sample using Ani Labsystems' corresponding kits. It is important to note that in this case the sample must be initially **diluted with the Mycoplasma pneumoniae or Bordetella pertussis sample diluent 1+100.** See details in corresponding kit instructions.

THE PROCEDURE:

STEP I
1. Pipette 100 µl of sample diluent into wells A1 and B1 (blanks).
2. Pipette in duplicates 100 µl of the ready for use controls (vials 3a and 3b) into the wells (C1 to F1) of the Microstrip®.
3. Pipette 50 µl of the diluted specimens (1:101) into Microstrip®.
4. Pipette 50 µl of sample diluent into each Microstrip® wells, except for A1 to F1 (final dilution of the sample 1:202). See note below. Cover the Microstrips® with plastic sheet.
5. Incubate for 1 hour (±5 min) at +37°C (±1°C).

WASHING
Washing may be performed manually or with a washer.

6. Empty the wells into a suitable biohazard container or aspirate the well contents with a washer.
7. Add 300-400 µl of washing solution into each well.
8. Empty the wells.
9. Repeat the washing cycle five times in total. After the washing step tap the inverted Microstrip® a few times on the paper towel.

STEP II
1. Pipette 100 µl of the conjugate (vial 4) into each well and cover the Microstrips® with plastic sheet.
2. Incubate for 1 hour (±5 min) at +37°C (±1°C).

NOTE: To avoid the contamination of the conjugate solution pour needed amount of the solution into a disposable reagent basin. Discard any unused conjugate solution, do not pour it back to the vial. For this purpose the kit includes 6 disposable reagent basins. Disposable reagent basins can be used also for the sample diluent and the TMB-substrate solution.

WASHING
Wash the wells five times in total as in items 6-9 in STEP I.

STEP III

1. Pipette 100 µl of the TMB-substrate solution (vial 5) into each well.
   Avoid contamination of the TMB-substrate solution: do not touch the walls of the wells with pipette tips when adding TMB-substrate.
   See NOTE below.
2. Incubate for 30 minutes at room temperature in a dark place.

STEP IV

1. Stop the enzyme-substrate reaction by adding 100 µl of stopping solution (0.45 M H_2SO_4, vial 6) into each well. Mix well. See NOTE below.

NOTE: The use of an 8-channel pipette device is recommended for improved efficiency and precision.

MEASUREMENT

Measure the absorbance immediately at 450 nm.

RESULTS

Quality Control Values

Before calculating the results, make sure that the absorbance values obtained for the reagent blank and controls fall within the Quality Control guidelines.

Bordetella pertussis positive and negative control is provided in each kit. The negative control and the positive control should be included in duplicate in each test run. Expected absorbance values for these controls are shown in Table. If the blank and the controls do not give expected values, the results are invalid and the specimens should be retested.

Note that both absorbance values of the duplicate measurement of positive control, negative control and reagent blank must fall within the expected values for the assay to be valid.

Quality control values

QC Sample	Expected value at 450 nm (in absorbance units)
Reagent blank	< 0.15
Negative control NC	< 0.40 *)
Positive control PC	0.80 ≤ PC ≤ 2.00 *)

*) = The reagent blank absorbance has already been subtracted from these values.

Calculation of the Results

Appreviations:

A = Absorbance
Arb = Mean absorbance of the reagent blank
Apc = Mean absorbance of the positive control
As = Mean absorbance of the sample
CO = Cut-off value in absorbance units

Use the following formula for calculating the **CO** (cut-off) and **S/CO** (signal to cut-off) values:

CO = 0.6 x (Apc - Arb)

S/CO = (As - Arb) / CO

Example:

Sample	Mean A at 450 nm
Reagent blank	0.030
Positive control	1.370
Sample 1	1.520

CO = 0.6 x (1.370 - 0.030) = 0.804

S/CO (Sample 1) = (1.520 - 0.030) / 0.804 = 1.85

Interpretation of the Results

S/CO < 0.5 — Negative.
A negative result indicates that the sample tested either contains no IgA antibodies to *Bordetella pertussis* or that the antibody level is not yet detectable. Sample may contain IgG or IgM antibodies to *Bordetella pertussis.*

0.5 ≤ S/CO <1.0 — Equivocal.
An equivocal result indicates that presence of *Bordetella pertussis* IgA antibodies can not be definitely established. In case of clinical suspicion retest antibodies after two weeks.

S/CO ≥ 1.0 — Positive.
A positive result may indicate either acute on-going or recent infection.

In addition, negative results with only one antibody class does not rule out possible infection. Thus all three classes should be measured. If one of the IgG **(see note below)**, IgA or IgM tests is positive, then the patient is most likely infected by *B. pertussis*.

Note: Special care should be taken when interpreting IgG positive results.

Vaccination program consists of three injections, usually at 0.5, 2 and 6 years of age. Before the first injection, a positive *B. pertussis* IgG result points towards *B. pertussis* infection. After teenage period, a high positive *B. pertussis* IgG result is suggesting *B. pertussis* infection. However, low positive (S/CO < 1.2) IgG should be confirmed on a second serum sample 2 to 4 weeks later.

During the vaccination program, the titer of IgG might be high depending on the vaccination status, and vaccination increases only the level of IgG antibody. In this case it is important to confirm infection with IgA and IgM antibody detection and preferably repeat the tests after 4 weeks with another serum sample.

If vaccination occurred less than 3 months ago, diagnosis should be performed by other laboratory methods.

LIMITATIONS OF THE PROCEDURE

Because no single method leads to the definitive diagnosis, the results of the present method should be interpreted in conjunction with the clinical condition and other laboratory methods.

PERFORMANCE CHARACTERISTICS

Reproducibility

Within run reproducibility

	replicates n	mean absor-bance	SD	CV %
B. pertussis IgA sample no 1	22	0.199	0.023	11.5
B. pertussis IgA sample no 2	22	1.317	0.033	2.5
B. pertussis IgA sample no 3	20	0.642	0.031	4.8
B. pertussis IgA sample no 4	20	1.295	0.043	3.3

Between run reproducibility

	repli-cates within-run	run n	mean abs	mean S/CO	SD S/C	CV %
B. pertussis IgA sample no 1	4	14	0.448	0.6	0.02	5.1
B. pertussis IgA sample no 2	4	14	0.548	0.7	0.03	6.9
B. pertussis IgA sample no 3	4	14	1.143	1.6	0.05	4.6
B. pertussis IgA sample no 4	4	14	0.145	0.2	0.01	9.0

Summary of the evaluation studies

Diagnostic specificity

The diagnostic specificity of the Ani Labsystems specific Bordetella pertussis IgG, IgA and IgM EIAs was compared to the results obtained from culture-negative and seronegative samples (from 0.5 to 72 years of age) tested during the clinical evaluation run in *Bordetella pertussis* reference laboratory in Finland. Results are summarized in Table1.

Table 1. Correlation between the Ani Labsystems Bordetella pertussis EIAs and negative reference samples.

Tests	Total samples	False positive	Specificity %
Ani Labsystems IgG EIA	161	8	95
Ani Labsystems IgA EIA	161	6	96
Ani Labsystems IgM EIA	161	11	93

The test is specific of *Bordetella pertussis* infection and does not cross react significantly with the other respiratory tract infectious disease tested. 54 positive samples for IgA and/or IgM antibodies against *Chlamydia pneumoniae* and 48 positive samples for IgA and/or IgM antibodies against *Mycoplasma pneumoniae* were tested with Ani Labsystems' Bordetella pertussis IgA and IgM EIAs. The specificity of Ani Labsystems' tests was 94 % with the *Chlamydia pneumoniae* positive samples and 96 % with the *Mycoplasma pneumoniae* positive samples.

Diagnostic sensitivity

No single antibody class detection alone nor single antigen used in detection will allow the exact diagnostic of *Bordetella pertussis* infection. Thus the whole cell based assay overcomes the pitfalls seen with the use of only one recombinant antigen. In addition, measurement of the three antibody classes (IgG, IgA and IgM) is strongly recommended for proper diagnosis and when ever applicable on paired sera. The tests were compared with 47 culture-proven positive samples. Out of 47 samples 28 were found positive after the first serum and 45 (96 %) after the second serum collection. These 96 % of those samples were found positive with at least one of Ani Labsystems IgG, IgA and IgM EIAS after the second serum illustrating the seroconversion of the patients during the delay between each serum.

Comparison with other tests

The performance of the Ani Labsystems' specific Bordetella pertussis IgG, IgA and IgM EIAs was compared to another commercial Bordetella pertussis IgG, IgA and IgM assay (using recombinant antigen)as well as to an in-house test (using whole cell antigen) performed by a clinical laboratory in Finland. The results are given as the percentage of concordant results between Ani Labsystems EIAs with at least one of the two other methods. Results are summarized in Table 2.

Table 2. Correlation between the Ani Labsystems Bordetella pertussis EIAs with another cell-based antigen assay and with a recombinant antigen-based assay.

Tests	Total samples	Concordant samples	Concordant results %
Ani Labsystems IgG EIA	92	84	91
Ani Labsystems IgA EIA	92	86	93
Ani Labsystems IgM EIA	92	84	91

TROUBLE SHOOTING

BLANK HAS TOO HIGH ABSORBANCE VALUES	
Cause/Error	**Remedy**
1. Contamination, spills from other wells	Avoid contamination

POSITIVE CONTROL HAS TOO LOW ABSORBANCE VALUES	
Cause/Error	**Remedy**
1. The control is not mixed with the sample diluent while pipetting	After dispensing the control rinse the pipette tip with sample diluent

NEGATIVE CONTROL HAS TOO HIGH ABSORBANCE VALUES	
Cause/Error	Remedy
1. Contamination, spills form other wells, interchange of the vial caps	Avoid contamination

POSITIVE CONTROL HAS TOO HIGH ABSORBANCE VALUES	
Cause/Error	**Remedy**
1. Incubation temperature is too high	Incubate at 37°C (± 1°C). The heating efficiency of incubators varies widely. The incubator with efficient and even warming is preferred
2.Incubation time is too long. This may be the case especially with automatic systems.	Check the real incubation times (should be 60 (±5) min, 60 (±5) min, 30 (±3) min)

ALL ABSORBANCE VALUES ARE VERY HIGH	
Cause/Error	**Remedy**
1. TMB-substrate solution is contaminated	Use clean containers
2. Washing solution concentrate has not been diluted correctly	Should be diluted 1:10 (1+9)
3. Poor washing	Check your washer
4. Contaminated solution containers	Use clean containers
5. Deterioration of reagents	Use aseptic technique. Do not pour used reagents back to vials

ALL ABSORBANCE VALUES ARE VERY LOW	
Cause/Error	**Remedy**
1. Incubation temperature is too low	Incubate at 37°C (± 1°C). The heating efficiency of incubators varies widely. The incubator with efficient and even warming is preferred.
2. Contamination of conjugate by human serum or plasma	One microliter of human serum/plasma is enough to inhibit as much as 1 liter of the conjugate. Never pour used conjugate back to vial!
3. The reagents have not been warmed up to room temperature	Should be +20°C - +25°C when starting the assay
4. Once opened microtiter plate foil package has not been resealed tightly and stored properly with desiccant	See instructions in REAGENT PREPARATION
5. TMB-substrate solution is exposed to direct sunlight	Avoid unnecessary exposure to light
6. Deterioration of reagents	Use aseptic technique. Do not pour used reagents back to vials

POOR PRECISION	
Cause/Error	**Remedy**
1. Liquid handling devices are not properly calibrated	Check calibration of the pipetting device
2. Improper washing due to contamination of washing tips head	Clean regularly tips of the washing head
3. The plate is allowed to stay too long after washing (drying of the plate)	Follow strictly the kit instructions
4. Uneven warming of the plate	Service the incubator in use
5. Sample serum/plasma is not mixed properly with sample buffer	While pipetting mix the sample with sample buffer
6. Stopping solution has not been mixed properly before measurement	Mix the plate before measuring

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RELATED PRODUCTS AND ORDER INFORMATION:

Product number	Product description
6111 500	Bordetella pertussis IgG EIA
6111 510	Bordetella pertussis IgA EIA
6111 520	Bordetella pertussis IgM EIA

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