ImmunoComb

Bovine Neospora Antibody Test Kit

INSTRUCTION MANUAL

Sufficient for up to 30/300 assays

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Biogal Galed Labs.

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Instruction Cat. No: 63BNC633

I. INTENDED USE OF THE KIT

This kit is designed to determine bovine plasma/serum or milk IgG antibody titers to *Neospora caninum*.

II. WHAT IS THE IMMUNOCOMB ASSAY?

The ImmunoComb is a self-contained portable kit. A sensitive test, which detects antibody levels in serum/plasma or milk. The ImmunoComb provides results within 38 minutes.

III. HOW DOES THE IMMUNOCOMB WORK?

- Based on a solid phase immunoassay principle, the ImmunoComb is a plastic card shaped like a comb, on which purified *Neospora caninum* antigen is attached.
- The ImmunoComb Kit contains 2 main components: a comb shaped plastic card, hereafter referred to as the Comb and a multi compartment developing plate.
- The Comb has 12 teeth – which may be used individually or any number up to 10, by breaking off the desired number of teeth from the Comb and using the corresponding column of wells in the developing plate. Each run should include Positive Control and Negative Control wells/teeth.
- Test spots of *Neospora caninum* are attached to each tooth on the Comb. The upper spot is the Internal Control, which indicate that the development is complete and valid (see example on section VII).
- The first step of the test is to deposit a serum, plasma or milk, in a well in row A of the multi-compartment developing plate.
- Next, the Comb is inserted into the well(s) with the sample(s) and transferred to the remaining wells (B-F) at timed intervals, according to the step by step instructions (see p. 5). Specific IgG antibodies from the specimen, if present, bind to the antigen at the test spots and will be labeled in row C, which contains an enzyme labeled anti-bovine IgG antibody.
- At the end of the developing process, a purple-grey color results are developed in all Internal Control spots (for validation only), in the Positive Control sample and in any positive sample tested spot.
- The intensity of the color result corresponds directly to the antibody level in the test specimen. Results are scored using the Positive Control spot and CombScale (see section VI).

IV. STEP BY STEP WITH IMMUNOCOMB

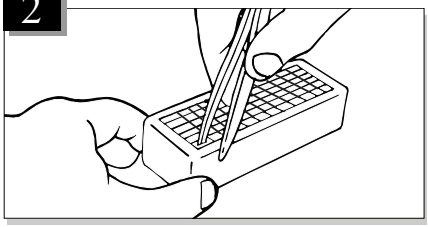
Before conducting the test, bring the developing plate to room temperature by removing all kit components from the kit carton and place them on the work bench for 60-120 minutes or incubate only the plate at 37°C/98.6°F for 25 minutes.

Perform assay at room temperature 20° – 25° C / 68° – 77° F.

1

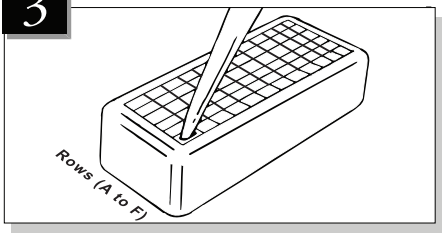
When testing serum/plasma use 5µl. When testing milk, use 100µl fresh sample

2



Mix reagents by gently shaking the developing plate several times prior to use.
Slit open the protective aluminum cover of row A with the tweezers. One well for each sample.

3



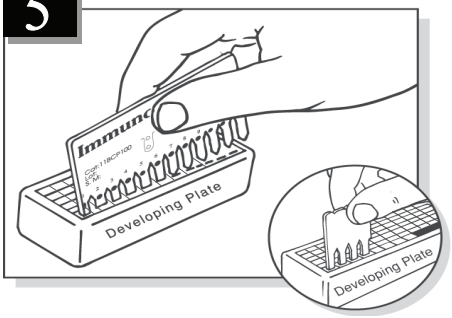
Dispense sample into each well. When using capillary tubes raise and lower the piston several times to achieve mixing. When using a pipette, mix by depressing the plunger a number of times.
Avoid spillage and cross-contamination of solutions.

4

Open the next 2 consecutive wells for control serum.
Take 5µl Positive Control serum (C+) and insert into well **A** next to the last sample. Mix the serum into the well. **Do the same with the Negative Control serum (C-) in the following A well.**

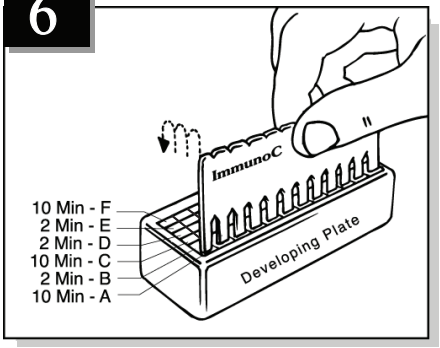
Do not open any wells of row A or other rows which you do not intend to use. Do not remove aluminum cover of developing plate all at once.

5

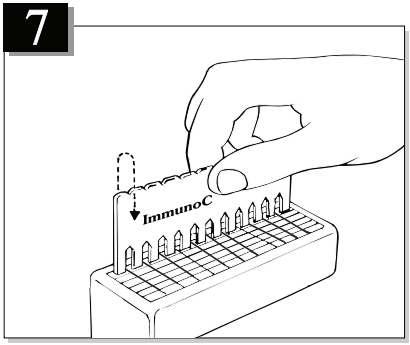


Remove one Comb from its protective envelope. Do not touch the teeth of ImmunoComb card. Insert the Comb (printed side facing you) into **Row A**. Incubate for **10 minutes**. **To improve mixing, gently move Comb up and down at the start of each incubation (each row). Repeat this motion at least twice in all of the remaining rows.** For using a partial Comb, cut the number of teeth needed including Positive and Negative Controls. Keep the remaining unused teeth sealed in its original envelope for further use. In each further step, open and use only the corresponding wells in the developing plate.

6



Pierce the cover of wells in **Row B** with the tweezers. Gently shake off excess liquid from the Comb onto a tissue (follow the same procedure for remaining rows at the end of each step). Insert Comb into wells of **Row B** and incubate for **2 minutes**, shake off and transfer the Comb to **Row C** and incubate for **10 minutes**. Place the Comb in **Row D** for **2 minutes**, **Row E** for **2 minutes**, and **Row F** for **10 minutes**, allowing the color reaction process to develop.

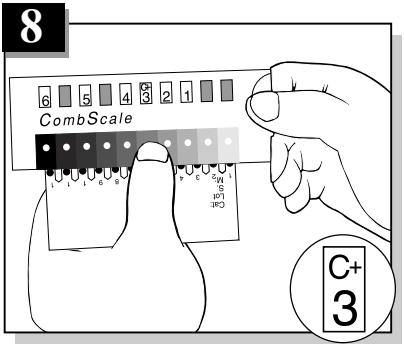


After the Comb has completed the cycle for **Row F**, transfer it back to **Row E**. Incubate in **Row E** for **2 minutes** for color fixation.

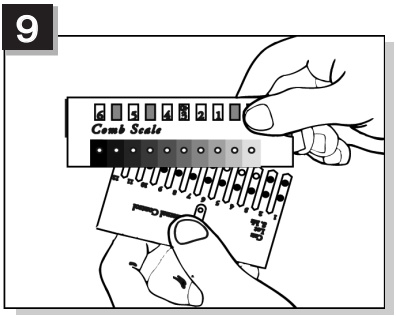
V. READING & INTERPRETING THE RESULTS

- Evaluate the results of each spot separately.
- The lower spot on the ImmunoComb tests for Neospora antibodies.
- Neospora IgG level is determined by comparing each specimen's color intensity to the Positive Control (C+), which is calibrated to 1:400 titer (IFA). To evaluate the antibodies score, use the CombScale provided in the kit (see section VI).
- Serum/plasma specimens with identical or higher color intensity than the Positive Control are considered positive. Milk samples are considered positive at cutoff scores of S2.
- The Negative Control consists of non-immune sera and should be read as zero (S=0). Sera with color intensity scored as S0 or S1 are considered negative. Milk specimens scored as S1, are considered suspicious.

VI. READING RESULTS USING THE COMBSCALE



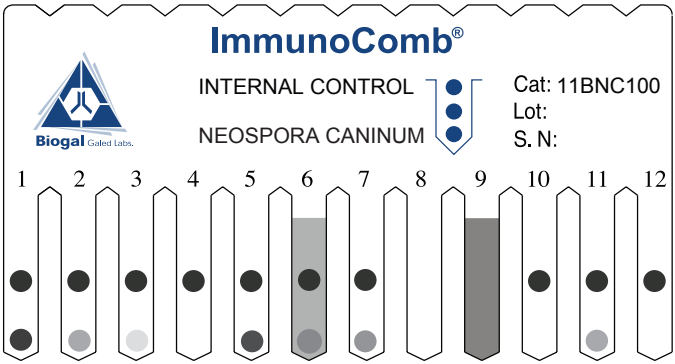
A. When the Comb is completely dry, align it with the calibrated color CombScale provided in the kit. Find the tone of the purple-grey on the CombScale that most closely matches the **Positive Control spot**. Slide the yellow ruler until the C+ mark appears in the window above the color you have found. **Hold the ruler in this position during the entire reading.** This step actually calibrates the C+ to S3, which is the "cut-off" point to which test spots will be compared.



B. While holding the ruler, find the tone of purple-grey on the CombScale that most closely matches the desired **test result spot** (one of the lower spots). The number that appears in the window above is the CombScale score (S0-S6).
Repeat this step with every test spot separately.

Important: The margin of errors is similar to that of other enzyme Immunoassay kit procedures. Therefore, an error in one color tone will not result in a wrong diagnosis.

VII. EXAMPLE OF A DEVELOPED COMB



TOOTH No.	RESULT & REMARKS
1,5	High positive reaction for Neospora.
2 ,7	Positive reaction for Neospora.
3	Low reaction for Neospora - Considered suspicious for serum /plasma. Considered positive in case of milk.
4,10	Negative reaction for Neospora.
6	High background with positive reaction.
8	No internal control - development failed.
9	High background color - invalid test.
11	Positive control.
12	Negative control.

For further assistance please contact your local Distributor, or Biogal Galed Labs. Acs. Ltd. directly by E-mail: info@biogal.com or by tel: 972-4-9898605 / fax: 972-4-9898690.

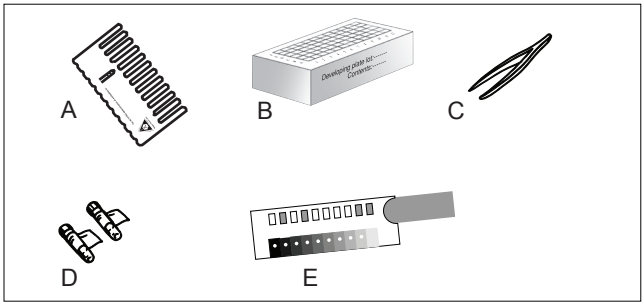
VIII. STORAGE & HANDLING

- Store the kit under normal refrigeration (2° – 8° C / 36° – 46° F). Do not freeze the kit.
- Do not mix reagents from different kits or from different compartments of the same kit.
- The ImmunoComb kit contains inactivated biological material. The kit must be handled and disposed of in accordance with accepted sanitary requirements.

IX. SAMPLE HANDLING AND STORAGE

- Fresh samples are recommended for use.
- Store serum and plasma samples at 2-8°C if the test is to be run within 3 days of collection. If test is delayed more than 3 days, freeze samples to -20°C or colder.
- Bring samples to room temperature and mix well before testing.

X. KIT CONTENTS



Componentes	30 Tests Kit (50BNC103)	300 Tests Kit (50BNC130)
A. ImmunoComb card (wrapped in aluminum foil)	3	30
B. Developing plate	3	30
C. Disposable tweezers	1	1
D. Positive and negative control	1 of each	1 of each
E. Calibrated CombScale	1	1
Instruction manual	1	1

XI. REFERENCES

Toolan (2002), *Neospora caninum* in cattle - a clinical perspective. I Vet J 56(8) 404-410.

Biogal's ImmunoComb