



[Reagent for food testing]

NH IMMUNOCHROMATO CAMPYLOBACTER

* Please read this manual carefully before using the kit.

Instruction Manual

[Introduction]

Campylobacter is known to have 17 species as of 2005. Among them, *Campylobacter jejuni* and *Campylobacter coli* are designated food poisoning bacteria ¹⁾.

Campylobacter food poisoning is extremely important for food hygiene, because in Japan, it has been one of the leading causes of food poisoning recently and ranked as top cause in 2007. The contamination with *Campylobacter* has been frequently observed in chicken, so it is necessary to not only prevent the secondary contamination from chicken, but also to take preventive measures at chicken farms and chicken processing factories ²⁾.

This product is a kit for detecting *Campylobacter* in foods by immunochromatography. Tests can be conducted rapidly and simply by means of the kit.

[Product Features]

- (1) The simple one-step operation of the kit.
- (2) The test gives rapid results.
- (3) There is no need for special test equipment.

[Kit contents]

1) Components

- | | | |
|----|--------------------|------------------|
| A: | Test plate | 5-test × 4 packs |
| B: | Instruction manual | 1 sheet |

2) Ingredients and Quantity

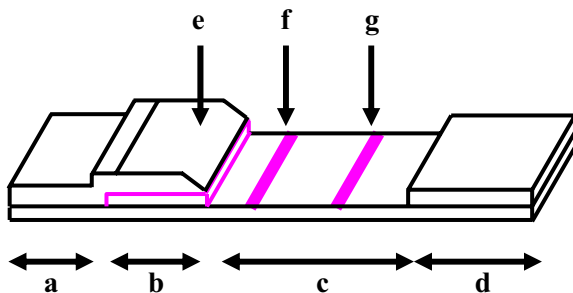
- | | | |
|----|--|---------|
| A: | Anti-Campylobacter monoclonal antibody (Mouse) | 0.38 µg |
| B: | Gold colloid labeled Campylobacter monoclonal antibody (Mouse) | 0.11 µg |
| C: | Anti-mouse immunoglobulin polyclonal antibody (Goat) | 0.38 µg |

[Application]

- 1) Detection of *Campylobacter* species (*C. jejuni* and *C. coli*) in foods

[Illustration of Test plate and the Principle of assay]

1) Illustration of Test plate



- a. Sample solution drop section
(Be careful not to touch this section with your finger.)
- b. Reagent-containing section
- c. Detecting section *(Be careful not to scratch this section and touch this section with your finger.)*
- d. Absorbent pad
- e. Measurement items listing position
- f. Test line appearance position
(Approx. 30mm from the sample solution drop section.)
- g. Control line appearance position
(Approx. 38mm from the sample solution drop section)

2) Principle of assay

When a sample solution is dropped onto the test sample drop section of the test plate, the gold colloid-labeled anti-*Campylobacter* antibody (2) in the test sample-containing section dissolves and forms complexes with *Campylobacter* (1) in the sample solution. These complexes move to the expanded section by capillary attraction and are trapped by the anti-*Campylobacter* antibody (3) that is fixed in the test line appearance position. This results in the appearance of a reddish purple line of gold colloid. This reddish purple line can be detected by visual inspection and used to judge the presence or absence of *Campylobacter*

in the test solution.

The excess gold-labeled antibodies, regardless of the presence or absence of *Campylobacter* in the test solution, travel further through the expanded section and are trapped by the anti-goat immunoglobulin rabbit antibody (4) fixed at the control line appearance position, where they form a second reddish purple line. The presence of this line indicates that the test solution has reached the expanded section.

[Preparation of the sample Solution]

*: The test solution preparation method is based on the protocol being reviewed at the “Food microbe standard test method review committee” (Protocol No. NIHSJ-02)³⁾ because no applicable notification has been published for the test method of *Campylobacter* in foods.

1) Required Equipment and Instruments

Stomacher bag (preferably with a filter), stomacher, incubator, autoclave, culture medium, microaerobic gas generation bag, and anaerobic jar, etc.

2) Preparation of Test Samples

- (1) Take a test sample of more than 200g of the food under test. In cases where surface contamination is suspected, the sample is taken by scraping off 300–500cm² of the surface to a thickness of 0.2–0.3mm.
- (2) Chop and mix the whole sample collected. Weigh 25g of the sample into the stomacher bag and use this as the test specimen

3) Sample enrichment

- (1) Add 100 mL of a Bolton medium to the 25-g specimen in the stomacher bag and homogenize with the stomacher for 30 seconds.
- (2) Incubate the specimen in the stomacher bag under a microaerobic condition at 35 ± 1°C for four hours and then at 42 ± 1°C for 24 to 44 hours.

Note1: Note 1: The basic microaerobic condition is defined as air consisting of 5% oxygen, 10% carbon dioxide gas, and 85% nitrogen.

Note2: It is also possible to enrich the sample by incubating it in a Bolton medium under a microaerobic condition at 42 ± 1°C for 24 to 48 hours or in a Preston medium under a microaerobic condition at 42 ± 1°C for 24 to 48 hours.

4) Sterilization

- (1) Remove the stomacher bag from the incubator. Gently mix the contents of the stomacher bag using a side-to-side motion, taking care not to splash it.
- (2) Transfer a necessary volume (basically, 0.5 to 1.0 mL) of the culture solution into another container using a sterilized pipette, and sterilize the solution by boiling (or heating at 95°C for five minutes in a heat block, etc.).
- (3) After the boiling sterilization, allow the container to cool to room temperature to prepare the sample solution.

Note1: Avoid catching specimen (food) residues in transferring the culture solution. If a large amount of residues are caught, centrifuge the culture solution at 3000 rpm for one minute and use the supernatant for the subsequent test procedure.

Note2: The kit can also detect viable *Campylobacter*, but tests with a culture solution sterilized by boiling are recommended to ensure the safety of the operator. When viable cells are used, the visibility of the detecting section is reduced due to the discoloration by blood components in the medium

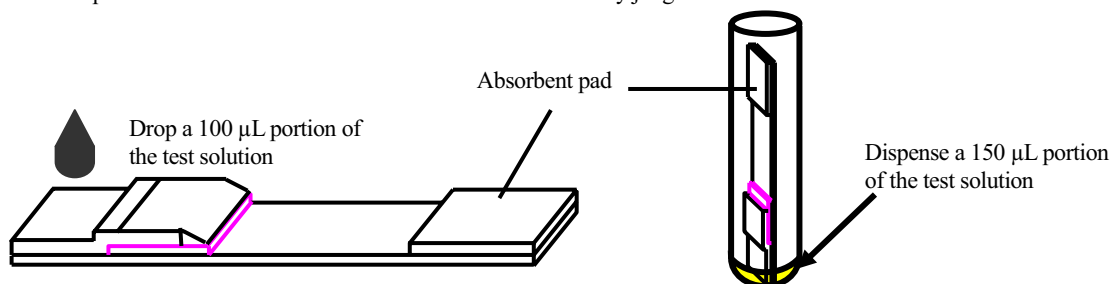
Note3: Do not autoclave the culture solution because autoclaving may reduce reactivity.

Note4: Because the remainder of the culture solution might be required for use in confirmatory tests following those conducted with the kit, do not sterilize it and retain it until all the tests have been completed.

[Operating Procedures for Testing]

1) NH Immunochromato *Campylobacter* Test Procedures

- (1) Bring the test plates contained in the aluminum pouch to room temperature and remove from the pouch as necessary immediately before use.
- (2) With an oil-based marker pen, write the name of the test sample or the number of the subject under test on the absorbent pad of the test plate removed from the bag.
- (3) Place the test plate carefully on the flat stand and drop a 100 µL-portion of the test solution onto the test sample drop section (see the figure on the left). Otherwise, dispense a 150 µL portion of the test solution into a test tube and attach the test plate to the test tube so that the test sample drop section of the test plate is immersed in the test solution (see the figure on the right).
- (4) Allow the test plate to stand undisturbed for 15 minutes and then visually judge.



Note1: Do not remove the test plate from the aluminum pouch until it has returned to room temperature, otherwise incorrect test results may be obtained as a result of moisture absorption.

Note2: Keep test plates that are not in use in a vinyl pouch containing desiccants and store this in an aluminum pouch in a refrigerator.

Note3: Be careful not to scratch the test sample drop section or expanded section and do not touch them with your hands. When

handling the test plate, make sure that you hold the absorbent pad.

Note4: Make sure that you use a sterilized pipette or chip to drop or dispense the sample solution. Change the pipette or chip for every test solution.

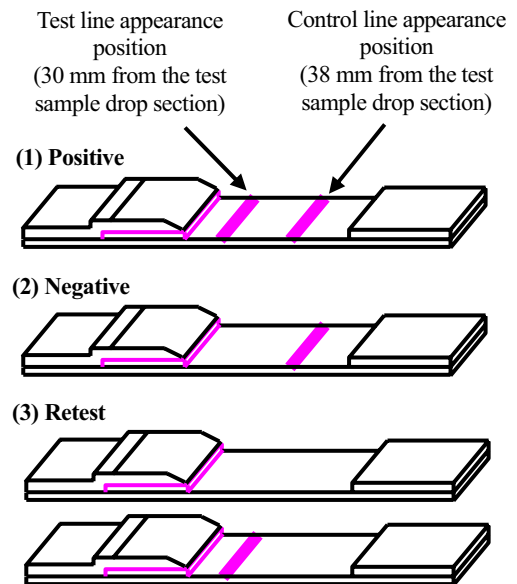
Note5: Make sure that the 150-µL portion of test solution does not overflow the test plate when dropping it. If necessary, drop the solution in two or more portions.

Note6: To prevent infection of the operator, it is recommended that the test plate should be placed on clear-plastic wrap, etc. when the test is performed by dropping the test solution.

3) Judgment of the Test Results

- (1) The test results is judged as positive when a reddish purple line is observed at the test line appearance position and at the control line appearance position 15 minutes after the start of the test.
- (2) Judge the test results as negative when no reddish purple line is observed at the test line appearance position, but a line is observed at the control line appearance position.
- (3) Retest in cases where no reddish purple line is observed at the control appearance position, regardless of the presence or absence of a line at the test line appearance position. It is likely that there is something abnormal in the development of the sample solution in such cases.

Note1: Ensure that confirmatory tests by other test methods are performed on specimens tested positive by the kit. The enriched samples used for testing with the kit can be used in the confirmatory tests.



[Performance]

1) Sensitivity Test

When the test is performed according to the “Preparation of the Sample Solution” and “Operating Procedures for Testing” in the manual, the test kit will show a positive result when *C. jejuni* is present at 1×10^5 CFU/mL or higher.

2) Repeatability Tests

When positive and negative test solutions of *C. jejuni* are simultaneously tested three times each, all positive test solutions exhibit positive results and all negative test solutions show negative results.

3) Minimum Detection Sensitivity

The minimum detection sensitivity of the kit was confirmed to be 1×10^4 to 10^6 CFU/mL in the test with six standard strains of *C. jejuni* and 1×10^5 to 10^6 CFU/mL in the test with two standard strains of *C. coli*.

Note1: The minimum detection sensitivity of this kit could vary depending on the effects of the components of the test solution.

4) Cross-reactivity

- (1) Cross-reactivity with the following bacterial strains has not been observed.

Strains	ID of standard strains	Results
<i>Campylobacter fetus</i> subsp. <i>fetus</i>	JCM2527	-
<i>Campylobacter fetus</i> subsp. <i>venerealis</i>	JCM2528	-
<i>Citrobacter freundii</i>	ATCC8090	-
<i>Enterobacter aerogenes</i>	ATCC13048	-
<i>Enterobacter cloacae</i>	ATCC13047	-
<i>Enterobacter sakazakii</i>	ATCC51329	-
<i>Escherichia coli</i> O157	ATCC43888	-
<i>Escherichia coli</i> O26	RIMD05091876	-
<i>Escherichia coli</i> O1	ATCC11775	-
<i>Escherichia hermannii</i>	JCM1473	-
<i>Klebsiella pneumoniae</i>	ATCC4352	-
<i>Klebsiella oxytoca</i>	ATCC8724	-
<i>Pseudomonas aeruginosa</i>	ATCC9027	-
<i>Proteus vulgaris</i>	ATCC6380	-
<i>Salmonella</i> Enteritidis	ATCC13076	-
<i>Salmonella</i> Typhimurium	ATCC13311	-
<i>Serratia liquefaciens</i>	ATCC27592	-
<i>Serratia marcescens</i>	ATCC8100	-
<i>Serratia odorifera</i>	ATCC33077	-

- (2) The test kit was found to be cross-reactive to *Campylobacter lari* and *Campylobacter upsaliensis* because they have the same antigen as *C. jejuni* and *C. coli*.

[Precautions in Using the Kit]

1) Precautions in Handling the Kit

- (1) Read the instruction manual carefully before use. Use the kit in accordance with the test method described in this manual.
- (2) Do not use a kit whose use-by date has passed. The expiry date is indicated on the label on the aluminum pouch.
- (3) The kit is a reagent designed to detect *Campylobacter* in foods. It is not to be used for clinical diagnosis.
- (4) Tests may give false-positive results due to the effect of ingredients in the specimen. Positive test results from the kit should be confirmed by other test methods.
- (5) Confirm with their manufacturers or distributors that any reagents (including culture media) required for preparation of test solutions and any instruments that are used are suitable for the purpose.
- (6) This instruction manual is intended as a guideline for those in charge of testing. Verify your own operating procedures for the kit and the appropriateness of its use for each particular food.
- (7) Product specifications may be changed without notice.

2) Precautions Regarding Risk Prevention

- (1) Even minute amounts of *Campylobacter*, which the kit is designed to detect, could cause infection. For this reason, and because there is a possibility of infection by microorganisms than other than *Campylobacter*, exercise full precautions in conducting tests by wearing protective gloves and safety glasses.
- (2) Tests should be performed only where appropriate equipment and facilities are available. Follow standard microorganism testing procedures under the guidance of responsible supervisors.
- (3) If you accidentally get any sample solution in your eyes or mouth, adopt emergency measures, such as immediately washing away the solution with tap water, and then seek medical attention.
- (4) If you feel unwell after performing a test with the kit, obtain immediate treatment from a physician.

3) Precautions Regarding Disposal of Waste Materials

- (1) Note that surplus test solutions and used test plates, culture media, and test samples could carry contagious microorganisms. Therefore, make sure that waste materials are subject to appropriate sterilization, for example by autoclave treatment for 20 minutes at 121 °C or immersion of the materials in a sodium chlorite solution for more than 1 hour.
- (2) Discard the kits, test samples, and surplus test solutions in strict compliance with your local waste-disposal regulations and with full consideration of environmental sanitation.

[Storage Method and Use-By Date]

- (1) Storage method: Refrigerate at 2–8 °C and shade from the light. Avoid freezing.
- (2) Use-by date: 12 months from the date of manufacture.

[Packaging Unit]

NH Immunochromato *Campylobacter* 20 tests

[References]

- 1) Ono K.: Modern media, Vol. 54, No. 5, 159-163 (2008)
- 2) Ito T., et al.: Veterinary and Animal Husbandry Journal, Vol. 60, No. 11, 911-915 (2007)
- 3) National Institute of Health Sciences, "Food microbe standard test method review committee"
<http://www.hihs.go.jp/fhm/kennsahou-index.html>

[Manufacturer]

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