DIAGNOSTIC AUTOMATION, INC.
21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA
Tel: (818) 591-3030 Fax: (818) 591-8383
onestep@rapidtest.com
technicalsupport@rapidtest.com
www.rapidtest.com

See external label	2°C-8°C	96 tests	REF 8321-3

Campylobacter Antigen Detection (In Food)

REF **8321-3**

INTENDED USE

The Diagnostic Automation, INC. *Campylobacter* assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen food products for the presence of thermophilic *Campylobacter* antigen.

SUMMARY

Infection by thermophilic *Campylobacter* species is a leading cause of human gastroenteritis. Of the various species of *Campylobacter*, *C. jejuni*, *C. coli* and *C. lari*, are the species most often associated with human illness. *Campylobacter* are often passed to humans through the handling or consumption of contaminated food, particularly foods of animal origin.

Recently, human infection with *Campylobacter* has been implicated in the induction of Guillain-Barré Syndrome (GBS) and reactive arthritis. GBS is a debilitating and potentially fatal neurological disease that produces paralysis.

Campylobacter species are gram negative, motile curved or spiral rods that require highly specialized growth conditions. Typical cultivation entails pre-enrichment and enrichment steps in broth, followed by isolation on a selective solid medium. Of particular importance in the cultivation of *Campylobacter* is the requirement for a microaerobic atmosphere.

Cultivation of thermophilic *Campylobacter* species from foods involves multiple media steps, and thus many days. The Diagnostic Automation, INC. C*ampylobacter* Microwell ELISA allows rapid testing (less than an hour) of the enrichment broth, thus eliminating the need for the plating and identification steps used in the standard methods. The Diagnostic Automation, INC. *Campylobacter* Microwell ELISA reduces time to result by several days.

PRINCIPLE OF THE TEST

The Diagnostic Automation, INC. *Campylobacter* ELISA is a double antibody (sandwich) ELISA utilizing specific anti-*Campylobacter* antibodies coated to microwells. After addition of the sample and the enzyme conjugate, a positive reaction (indicating the presence of *Campylobacter* antigen) produces a deep blue color. Addition of the Stop Solution ends the assay and turns the blue color to yellow. The results may be read visually or with an ELISA reader.

REAGENTS

- Microwell test strips containing anti-*Campylobacter* polyclonal antibodies: 96 Test Wells
- Test strip holder: One (1)
- Enzyme Conjugate: One (1) bottle containing 11 ml of peroxidase conjugated anti-*Campylobacter* polyclonal antibody with red dye and a preservative.
- Positive control: One (1) vial containing 2 ml of *Campylobacter* antigen in a buffered base.
- Negative control: One (1) vial containing 2 ml of buffered base.
- Chromogen: One (1) bottle containing 11 ml of chromogen tetramethylbenzidine (TMB).
- Wash Concentrate 20X: Two (2) bottle containing 25 ml of concentrated buffer and surfactant with preservative.
- Stop solution: One (1) bottle containing 11 ml of 1 M phosphoric acid.

Additional Materials Required:

- Incubator (shaking preferred) capable of microaerobic atmosphere conditions (35-42 °C)
- Microelisa plate reader capable of bichromatic reading at 450/620-650 nm (optional)
- Pipettor, 100 µl
- Disposable micropipette tips
- Microbiological media and antibiotics for preparation of necessary enrichment broths
- Appropriate containers for storage and disposal of materials potentially contaminated with infectious agents
- Data record sheets
- Disinfecting Solution
- Boiling Water Bath
- Capped tubes for heating samples

PRECAUTIONS

Do not use solutions if they precipitate or become cloudy.
Exception: Wash concentrate may precipitate during refrigerated storage but will dissolve upon warming.

Do not add azides to the samples or any of the reagents.
Some reagents contain a preservative.
Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
Though and complete washing steps is critical to proper performance of the test.

STORAGE CONDITIONS

Reagents, strips and bottled components: Store between 2 -7 °C.
Squeeze bottle containing diluted wash buffer may be stored at room temperature.

REAGENT PREPARATION

Wash Buffer - Remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

MEDIUM PREPARATION

Bolton's Media: Acumedia catalog #7526 or equivalent - Follow manufacturer's instructions.

SAMPLE PREPARATION

Follow procedures listed in the Food and Drug Administration Bacteriological Analytical Manual (BAM- reference #5).
Boil samples for 10 minutes and allow to cool before testing.

TEST PROCEDURE

1. Break off the required number of wells (number of samples plus 2) and place in strip holder.
2. Add 100 µl of the negative control to well #1 and 100 µl of the positive control to well #2.
3. Add 100 µl of the test sample to the appropriate well.
4. Incubate at room temperature (15 to 25 °C) for 30 minutes, then wash.*
5. Add 2 drops of Enzyme Conjugate (red solution) to each well.
6. Incubate for 15 minutes, then wash. Slap out excess fluid against an absorbent towel.
7. Add 2 drops of Chromogen to each well.
8. Incubate for 5 minutes.
9. Add 2 drops of stop solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
10. Read results visually or at 450/620-650 nm.

* Each washing consists of dumping the contents of the wells into an appropriate container with disinfecting solution (e.g. 3% bleach in water) and using the diluted wash buffer to fill in each well, shaking out the contents and refilling the wells for a total of 3 times. Samples with sticky particulate matter may require more thorough washing than other samples. The potential exists for false positive results if the sample is not thoroughly washed from the well before addition of subsequent reagents.

Only one set of controls is required per run.
Read results within 4 hours from addition of Stop Solution.
All incubations are at room temperature (15-25 °C).

INTERPRETATION OF RESULTS - VISUAL

Positive: Any sample well that has significant and obvious yellow color.
Negative: Any sample well that does not have significant and obvious yellow color.

NOTE: The negative control, as well as some samples, may show some slight color. A sample well must be obviously darker than the negative control well to be called a positive result.

INTERPRETATION OF RESULTS – OD READINGS

Zero reader on air. Read all wells using a bichromatic reading with filters at 450nm and 620-650nm.

Positive: Add 0.10 OD units to the value of the negative control. Sample OD values of 0.10 above the negative control indicates the sample contains *Campylobacter* antigen.
Negative: Absorbance reading less than 0.10 plus the negative control indicates the sample does not contain detectable levels of *Campylobacter* antigen.

QUALITY CONTROL

The Positive and Negative Controls must be run each time the assay is performed.
For a valid run, the Negative Control must be below 0.10 ODs and the Positive Control greater than 0.5 OD units. If either Control is out of range, do not use the kit and contact Diagnostic Automation, INC. Technical Service at (818) 591-3030.

Problem: Negative control has substantial color development.
Correction: Washings were insufficient. Repeat test with more vigorous washings.

REFERENCES

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2. Nachamkin, Irving, 1997. Microbiologic Approaches for Studying *Campylobacter* Species in Patients with Guillain-Barré Syndrome. J of Infect Dis Vol 176 (Suppl 2) pp. S106-114
3. Stern, Norman J. et. Al. 19XX. Campylobacter – Chapter 29. Indicator Microorganisms and Pathogens. Pp. 475-495
4. Bolton, FJ and Robertson, L. 1982. A Selective medium for isolating *Campylobacter jejuni/coli*. J Clin Pathol Vol 35, pp. 462-467.
5. BAM 1998

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