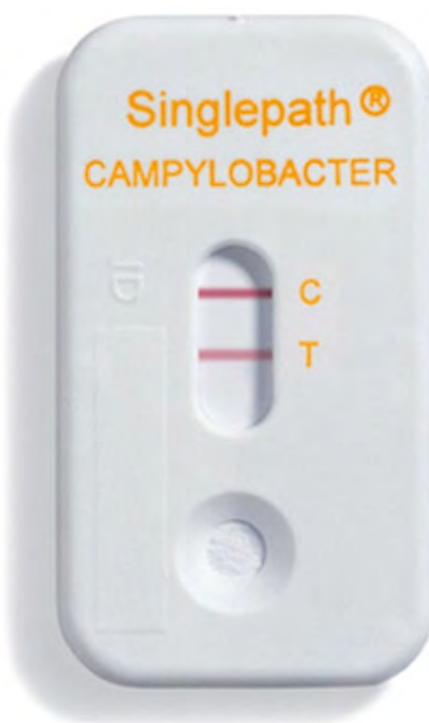


ЭКСПРЕСС-ТЕСТ SINGLEPATH CAMPYLOBACTER



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Singlepath® Campylobacter

GLISA-Rapid Test (Gold Labelled ImmunoSorbent Assay) for the qualitative detection of **Campylobacter spp** in food.

Introduction

Campylobacter spp. are now the leading cause of human enteritis in both the western world and developing countries. Recent infection with certain **C.jejuni** strains has also been associated with the debilitating neurological disorder, Guillain-Barre Syndrome (GBS) and reactive arthritis.

Campylobacter spp. are components of the intestinal flora of a wide range of wild animals and birds, farm animals and domestic pets. Human infection is mainly acquired from consumption of contaminated undercooked food, essentially, meats, poultry, shellfish and unpasteurised milk. Also, less commonly, some fruit and vegetables. However, infection can also be acquired from the environment. Water can become contaminated with animal and avian faeces, agricultural run-off and sewage effluent.

Campylobacter spp. are highly infective: as few as 500 bacteria are required to cause illness. **C.jejuni** and **C.coli** are the most common causative agents of human enteritis. **C.lari** and the emerging human pathogen, **C.upsaliensis**, have also been reported in a small percentage of cases. **C.fetus** infection is more rare, mainly systemic, especially in immunocompromised patients, and has been associated with abortion in humans.

The majority of Campylobacter spp. have low biochemical activity, therefore, identification is difficult on phenotypic characteristics. The standard detection method is enrichment for 48 hours in a microaerophilic environment, followed by isolation on selective agars for 48 hours in a microaerophilic environment. Results are therefore only available after 4 - 5 days.

The Singlepath® Campylobacter test, however, greatly reduces the time-to-result. Following 48 hour enrichment, a result is obtained on the heat-killed sample within 20 minutes, thereby eliminating the isolation step. The need for microaerophilic enrichment can also be eliminated if the Sample Enrichment Protocol below is followed.

Singlepath® Listeria is an immunological screening test based on the immune flow principle and is designed in such a way that time-consuming and personnel intensive working steps are avoided.

Mode of Action

Singlepath® Campylobacter is an immunochromatographic rapid test based on gold-labelled antibodies. The test device has a circular sample port, and an oval shaped test (T) and control (C) window.

1. The sample is applied to the chromatography paper via the circular sample port.
2. The sample is absorbed through the pad to the reaction zone containing colloidal, gold-labelled antibodies specific to Campylobacter spp.
3. Any Campylobacter antigen present complexes with the gold-labelled antibody and migrates through the port until it encounters a binding zone in the test (T) area.
4. The binding zone (T) contains another anti-Campylobacter antibody, which immobilises any Campylobacter-antibody complex present. Due to the gold-labelling, a distinct red line is then formed.

-
5. The rest of the sample continues to migrate to a second binding reagent zone within the control (C) zone, and also forms a second distinct red line (positive control). Regardless of whether any *Campylobacter* is present or not, this distinct red line is always formed in the control (C) zone, thus ensuring the test is working correctly.
 6. The rest of the sample continues to migrate to a second binding reagent zone within the control (C) zone, and also forms a second distinct red line (positive control). Regardless of whether any *Listeria* is present or not, this distinct red line is always formed in the control (C) zone, thus ensuring the test is working correctly.

Storage / Stability

Singlepath® *Campylobacter* is stable until the expiry date printed on the box, when stored at 2 to 8°C.

Sample Material / Sample Enrichment

- Add 25 g solid sample to 225 ml Bolton enrichment broth in a 250 ml Polystyrene Bottle or 25 ml liquid sample to 200 ml Bolton enrichment broth in a 250 ml Polystyrene Bottle.
- If necessary, transfer to the filter unit of a Stomacher bag and homogenise in Stomacher for 1 minute.
- Transfer homogenate back to 250 ml Polystyrene Bottle, ensuring a headspace of 10 - 15 % is provided (see Note below).
- Discard the Stomacher bag and filter unit.
- Incubate for 4 h at 37 °C.
- Transfer to 41.5 °C and incubate for a further 44 h.

Note: If headspace is more than 15%, incubate enrichment culture microaerophilically, using a controlled atmosphere chamber, "Anaerocult" C gas packs (1.16275. or 1.13682.) can be used to generate this.

Experimental Procedure and Evaluation

Sample Preparation

1. Transfer approx. 1-2 ml enrichment culture to an appropriate (polypropylene) tube. Cover with a loose-fitting cap.
2. Place tubes in boiling water bath for 15 min.
3. Remove and allow cooling to room temperature (18 - 26 °C), prior to use.

Allow test devices to warm to room temperature if stored at 2 to 8 °C.

Procedure

1. Remove the foil pouches from the required number of Singlepath *Campylobacter* devices. Place the test device(s) on a flat surface and label with appropriate sample identification. (Note: Perform the tests within a period of 2 hours after opening!)
2. Using a disposable transfer pipette, draw up a sample from the boiled, cooled enrichment. If the broth contains sediment, such as horse blood, RESUSPEND the sediment before taking out the sample.
3. Dispense five (5) free falling drops (about 150 - 160 µl) into the circular sample port on the test device. Alternatively using a micro pipette and disposable pipette tip, dispense 160 µl sample into the circular sample port on the test device.
4. Observe the test result 20 minutes after applying the sample to the device.

Interpretation of Results

The test can be regarded as working correctly if a distinct red line appears in the control zone (C) within 20 minutes.

A sample can be considered POSITIVE if at or prior to 20 minutes, distinct red lines appear on both test (T) **and** control (C) zones.

A sample can be considered NEGATIVE if no red line appears in the test (T) zone but does appear distinctly in the control (C) zone 20 minutes after application of sample to the device.

Technical Specifications

Detection limit

Depending on serogroup, a range of approx. 10^4 - 10^7 bacteria/ml can be regarded as being the lower detection limit.

Interferences

Results obtained to date on numerous food samples indicate that there is no interference of Singlepath® Campylobacter with food ingredients.

The test has been developed based on using Bolton enrichments. Interference from other types of enrichment broth and other brands of Bolton broth cannot be excluded.

Sensitivity	98 %
Specificity	100 %

Trouble-shooting

Problem	Measures
No line appears in either zone after 20 minutes test period	If sediment has accidentally been deposited in the sampling well, try carefully scraping this off using a disposable inoculation loop
	If unsuccessful, re-run sample avoiding sediment when sampling.
Delay in sample reaching Nitrocellulose	Touch sample pad with pipette tip

Ordering Information

Product	Ordering No.	Pack size
Singlepath® Campylobacter	1.04143 .0001	25 tests
Anaerocult® C	1.16275.0001	1 x 25
Anaerocult® C mini *	1.13682.0001	1 x 25
Bolton Broth	1.00068.0500	500 g
Bolton Broth Selective Supplement	1.00079.0001	16 vials
Campylobacter blood free Selective agar Base (modified CCDA-Preston)	1.00070.0500	500 g
CCDA Selective Supplement	1.00071.0001	16 vials
Lysed Horse Blood		

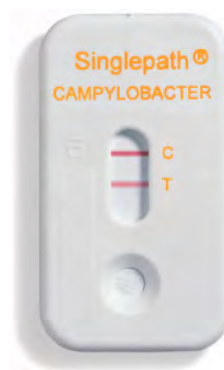
* Optional

Additionally required materials and instrumentation

- Disposable 250 ml Sterile Polystyrene Bottles with flow-seal cap for enrichment
- Stomacher / Stomacher bags with net-lined inserts
- 5 ml disposable tubes; 2 ml and 200 ml disposable pipettes
- Incubators 37 °C and 41.5 °C
- Distilled or de-ionized water
- Waterbath for boiling of samples
- Disposable Polypropylene tubes for boiling of samples
- Disposable plastic transfer pipettes and/or appropriate micro pipettes and disposable tips for dispensing 1-2 ml (sample for boiling) and 160 µl (application of oiled sample onto tests)
- Autoclave



Singlepath® Campylobacter Test
result negative



Singlepath® Campylobacter Test
result positive

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