

Qualitative detection

Qualitative detection		
Step	NMKL 119, rev.	ISO 10272-1:2006(E)
<b>Enrichment in selective liquid medium</b>	<p><i>With pre-incubation:</i> 25 g of sample is transferred to 225 ml Bolton basal broth with lysed blood. Incubation: In microaerobic atmosphere at 37 °C ± 1 °C for 2-4 h and then the selective supplement is added. Incubation is continued at 41,5 °C ± 1 °C for 48 h ± 4 h</p> <p><i>Without pre-incubation:</i> 25 g of sample is transferred to 225 ml Bolton complete broth. Incubation: In microaerobic atmosphere at 41,5 °C ± 1 °C for 48 h ± 4 h</p>	<p>x g or x ml test sample is transferred to 9x g or 9x ml Bolton broth (test portion/enrichment media ratio 1:10) and homogenized. Incubation: In microaerobic atmosphere at 37 °C for 4 h to 6 h and then at 41,5 °C for 44 h ± 4 h</p>
<b>Isolation and selection for confirmation</b>	<p>Approx. 10 µl of inoculum is transferred onto the surface of a mCCDA plate or an AHB (+TTC) agar plate. Incubation: In microaerobic atmosphere at 41,5 °C ± 1 °C for 48 h ± 4 h. Plates are inspected for visible growth.</p>	<p>From the cultures two selective solid media are inoculated: mCCD agar and any other selective medium based in a principle different from that of mCCD agar Incubation: In microaerobic atmosphere at 41,5 °C for 44 h ± 4 h. Plates are inspected to detect the presence of colonies presumed because of their characteristics to be <i>Campylobacter</i></p>
<b>Confirmation</b>	<p>Five <i>Campylobacter</i>-like colonies are subcultured in a non-selective agar (blood agar base no 2, Mueller-Hinton agar or any other suitable non-selective media. Incubation: In microaerobic atmosphere at 41,5 °C ± 1 °C for 24 h ± 3 h.</p> <p><u>Morphology and motility:</u> Suspend material from a suspect colony in a rich broth (nutrient broth, Brain Heart Infusion or similar) and examine morphology and motility using a microscope.</p> <p>If further identification is required:</p> <p><u>Oxidase test:</u> Use fresh colony material from non-selective agar and oxidase reagent. Positive reaction is seen within 20-30 s.</p> <p><u>Catalase test</u> <u>Hydrolysis of hippurate</u> Hydrolysis of indoxylacetate</p>	<p>At least one colony presumed to be <i>Campylobacter</i> is subcultured on non-selective Columbia blood agar and further four colonies if the first is negative. Incubation: In microaerobic atmosphere 41,5 °C for 24 h to 48 h.</p> <p><u>Morphology and motility:</u> Suspend one colony from the Colombia blood agar plate in 1 ml Brucella broth and examine morphology and motility using microscope.</p> <p><u>Study of growth at 25 °C (microaerobic) and 41,5 °C (aerobic):</u> Inoculate colonies from the Colombia blood agar plate onto two Colombia blood agar plates. Incubate one plate at 25 °C in microaerobic atmosphere for 44 h ± 4 h. Incubate the other plate at 41,5 °C for 44 h ± 4 h.</p> <p><u>Oxidase test:</u> A portion of colony from the Colombia blood agar plate is streaked onto a filter paper moistened with oxidase reagent. Positive reaction is seen within 10 s</p> <p><u>Optional identification of <i>Campylobacter</i> species:</u> Catalase test, detection of sensitivity to nalidix acid and to cephalothin, detection of hippurate hydrolysis and detection of indoxyl hydrolysis.</p>
<b>Expression of results</b>	<p>Thermotolerant <i>Campylobacter</i> present or not present in the amount of sample analysed</p>	<p>The presence or absence of <i>Campylobacter</i> in a test portion of x g or x ml of product</p>

**Quantitative detection**

	<b>Quantitative detection</b>	
<b>Step</b>	<b>NMKL 119, rev.</b>	<b>ISO 10272-2:2006(E)</b>
<b>Test portion, initial suspension and dilution</b>	10 g of sample is stomached with 90 ml Buffered Peptone Water (BPW) for 30 seconds. From this dilution ( $10^{-1}$ ) a 10 fold serial dilution is made on BPW	Prepare a single decimal dilution series (see ISO 6887 and ISO 8261) from the test sample if the product is liquid, or from the initial suspension in the case of other products (diluent see ISO 6887). Make serial 10 fold dilutions if necessary
<b>Inoculation and incubation</b>	0,1 ml inoculum from each dilution is spread onto the surface of one mCCDA or AHB (+TCC) plate. Incubation: $41,5 \pm 1,0^{\circ}$ C for $48 \pm 4$ h in microaerobic atmosphere	0,1 ml inoculum from each dilution is spread to each of two plates of mCCD plates. Incubation: $41,5^{\circ}$ C for 40 h to 48 h in microaerobic atmosphere
<b>Counting and selection of colonies for conformation</b>	Count colonies at plates containing 10-100 colonies. Choose five suspect colonies for subculturing for the confirmation tests. Streak each selected colony onto a non-selective agar plate (blood agar base no 2, Mueller-Hinton agar or any suitable non-selective media). Incubation: $41,5 \pm 1,0^{\circ}$ C for $24 \pm 3$ h in microaerobic atmosphere	Count colonies at plates containing less than 150 typical or suspect colonies. Choose at random five such colonies for subculturing for the confirmation tests. Streak each selected colony onto a Colombia blood agar plate. Incubation: $41,5^{\circ}$ C for 24 h to 48 h in microaerobic atmosphere
<b>Confirmation of <i>Campylobacter</i> species</b>	<u>Morphology and motility:</u> Suspend material from a suspect colony in a rich broth (nutrient broth, Brain Heart Infusion or similar) and examine morphology and motility using a microscope.  If further identification is required:  <u>Oxidase test:</u> Use fresh colony material from non-selective agar and oxidase reagent. Positive reaction is seen within 20-30 s.  <u>Catalase test</u> <u>Hydrolysis of hippurate</u> <u>Hydrolysis of indoxylacetate</u>	<u>Morphology and motility:</u> Suspend one colony from the Colombia blood agar plate in 1 ml Brucella broth and examine morphology and motility using a microscope. <u>Study of growth at <math>25^{\circ}</math> C (microaerobic) and <math>41,5^{\circ}</math> C (aerobic):</u> Inoculate colonies from the Colombia blood agar plate onto two Colombia blood agar plates. Incubate one plate at $25^{\circ}$ C in microaerobic atmosphere for 40-48 h. Incubate the other plate at $41,5^{\circ}$ C for 40-48 h. <u>Oxidase test:</u> A portion of colony from the Colombia blood agar plate is streaked onto a filter paper moistened with oxidase reagent. Positive reaction is seen within 10 s.
<b>Expression of results</b>	Number of thermotolerant <i>Campylobacter</i> per g sample	Number of <i>Campylobacter</i> per ml or g product