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Microbiology
Bactident® E. coli

Rapid identification kit for E. coli

Contents:

50 test strips
50 reaction vessels
1 tray for holding the reaction vessels
1 dropping bottle filled with KOVACS' reagent (5 ml)

Composition:

The reaction zone of one Bactident® E. coli test strip contains:

4-Methylumbelliferyl-β-D-glucuronide	3.0 nmol
L-Tryptophan	0.4 µmol
Buffering agents	
Detergent	

The KOVACS' reagent contains (mol/l):

Dimethylaminobenzaldehyde	0.07
N-Butanol	1.6
Hydrochloric acid, 37%	0.6

Principle:

E. coli is identified by detection of the enzymes β-D-glucuronidase and tryptophanase (indole formation). β-D-glucuronidase activity is a specific marker for E. coli as far as the Enterobacteriaceae family is concerned.

94 % of all E. coli strains posses this enzyme whereas only a few Salmonella and Shigella species display a positive β-D-glucuronidase reaction. The test for the formation of indole from tryptophan is positive in 99 % of all E. coli strains.

In the present test strips the substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) is degraded by β-D-glucuronidase. The presence of this enzyme is indicated by the appearance of light blue fluorescence in UV light (360 nm).

Indole formation is indicated if the suspension becomes red on addition of KOVACS' reagent.

Preparation:

Suspend a single, well-developed colony in 0.2 ml deionized water, the resulting suspension should be opalescent (MacFarland standard 2).

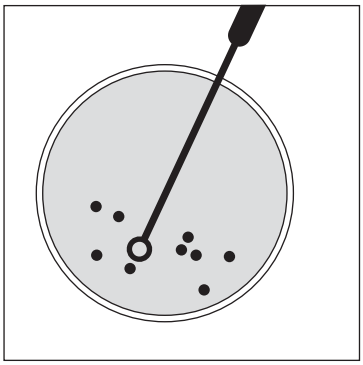
Stability: see expiring date.

Only remove the amount of strips needed at the time! Do not touch the reaction zones of the test strips. Close receptacle firmly immediately after use. Please store at the specified temperature.

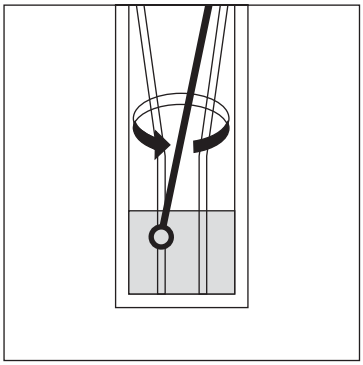
Safe removal:

Test strips and reaction vessels are to be removed safety after use like bacteria containing material. This may be done by burning, autoclaving or by placing into a 5 to 6 % disinfectant solution – for at least 6 hours.

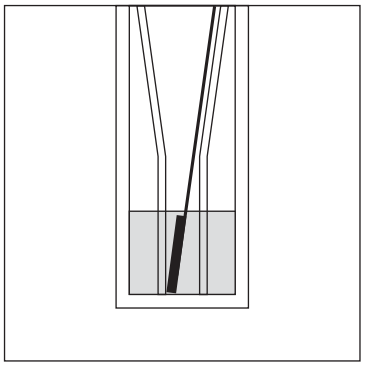
Procedure:



1.
Remove isolated, well-developed colonies from the culture medium with a plantinum loop (fig. 1).

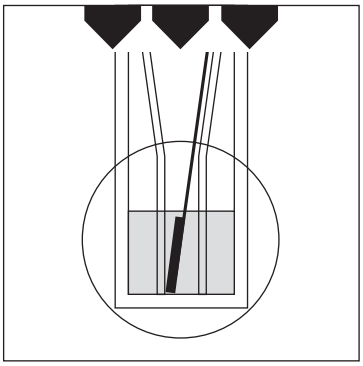


2.
Suspend the bacterial mass in a reaction cuvette containing 200 µl deionized water and place the reaction vessel in the tray (fig. 2).

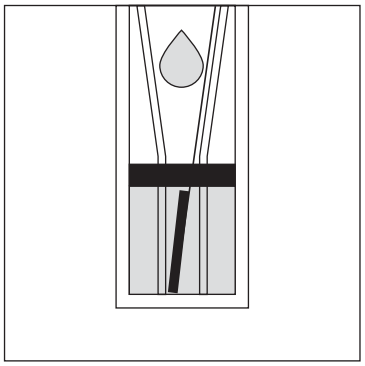


3.
Insert the test strip in the tapered slit in the reaction vessel. The groove holds the strip in the middle of the reaction chamber (fig. 3).

4.
Incubate at 37 °C for 30–120 minutes, depending on the culture medium used (see Table 1).



5.
Assess the reaction under a longwave UV lamp (about 360 nm). Blue fluorescence indicates the presence of β-D-glucuronidase (fig. 4).



6.
In order to detect indole formation, add one drop of KOVACS' reagent to the cuvette (from the dropping bottle). The appearance of a red colouration after 1–2 minutes signifies a positive reaction (fig. 5).

Table 1:

β-D-glucuronidase-activity of E. coli (ATCC 25922) after cultivation on various culture media

Culture medium	Incubation period [min]			
	30	60	90	120
Blood agar	+	++	++	++
Chocolate agar	+	++	++	++
CASO-agar	+	++	++	++
Standard I nutrient agar	+	++	++	++
Standard II nutrient agar	+	++	++	++
BROLACIN agar	–	(+)	++	++
MacCONKAY agar	–	–	+	++
HEKTOEN enteric agar	–	–	(+)	+
EMB agar	–	–	(+)	+
XLD agar	–	–	+	++
Deoxycholate lactose agar	–	–	+	++

Enzyme activity: – negative
+ positive – clear fluorescence
++ positive – very strong fluorescence

Note:

The reaction time depends on the culture medium used. If colonies that have grown on a culture medium low in carbohydrate (blood agar, chocolate agar, CASO agar, standard I agar, standard II agar) are tested, the reaction can be checked after an incubation period of 30–60 minutes. In the case of colonies isolated on a culture medium with a high carbohydrate content (MacCONKEY agar, HEKTOEN enteric agar, XLD agar, deoxycholate lactose agar), an incubation period of 90–120 minutes is required.
The indole reaction may be weak or negative with the used incubation times in those cases the culture media contain acid hydrolyzed casein hydrolysate as the only peptone (MUELLER-HINTON-Agar).

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