



# ENTEROSYSTEM 18R

System for the identification of Gram-negative, oxidase negative enterobacteria

## DESCRIPTION

**ENTEROSYSTEM 18R** is a 18-well system containing desiccated biochemical substrata for the identification of Gram-negative, oxidase negative enterobacteria.

The system is inoculated with the suspension of the organism to be examined and incubated at  $36\pm 1^\circ\text{C}$  for 12-18-24 hours. The microorganism is identified by assessing the colour change of the various wells in order to determine the corresponding numerical code.

## CONTENT OF THE PACKAGE

Ref. 71618	Ref. 79618
<ul style="list-style-type: none"> <li>• 20 ENTEROSYSTEM 18R</li> <li>• 20 Vials of Physiological Solution (7.0 mL)</li> <li>• Instruction sheet and Data chart</li> </ul>	<ul style="list-style-type: none"> <li>• 4 ENTEROSYSTEM 18R</li> <li>• 4 Vials of Physiological Solution (7.0 mL)</li> <li>• Instruction sheet and Data chart</li> </ul>

## ITEMS NECESSARY BUT NOT INCLUDED IN THE PACKAGE

ENTEROSYSTEM 18R Reagent (ref. 80252): vaseline oil, indole test reagent, VP test reagents	
GRAM COLOR KIT (ref. 80293)	ENTEROSYSTEM 18R Code book (ref. 71710)
OXIDASE TEST STICK (ref. 88029)	Identification Code Disk (ref. 71711)

## PRINCIPLE OF THE METHOD

**ENTEROSYSTEM 18R** allows the identification of Gram-negative, oxidase negative enterobacteria of clinical significance. Identification is based on biochemical tests performed in the wells containing specific culture media. The combination of positive and negative reactions allows to determine a numerical code that in turn permits to identify the examined bacterium by using the ENTEROSYSTEM 18R Code Book (ref. 71710) or the Identification Code Disk software (ref. 71711).

## CONFIGURATION

Well	BIOCHEMICAL REACTIONS FOR MICROBIAL IDENTIFICATION
1-ONPG	Hydrolysis of ONPG (Ortho-Nitrophenyl- $\beta$ -Galactoside)
2-LDC <input type="checkbox"/>	Decarboxylation of lysine
3-ODC <input type="checkbox"/>	Decarboxylation of ornithine
4-ADC <input type="checkbox"/>	Decarboxylation of arginine
5-PD	Deamination of phenylalanine
6-CIT	Utilization of citrate
7-UR <input type="checkbox"/>	Hydrolysis of urea
8-H <sub>2</sub> S <input type="checkbox"/>	Production of hydrogen sulphide
9-MLN	Utilization of malonate
10-VP *	Production of acetoin (Voges-Proskauer test)
11-IND *	Production of indole (Kovac's test)
12-GLU	Fermentation of glucose
13-MAN	Fermentation of mannitol
14-INO	Fermentation of inositol
15-SOR	Fermentation of sorbitol
16-SAC	Fermentation of saccharose
17-ARA	Fermentation of arabinose
18-RAF	Fermentation of raffinose

: after inoculation, add vaseline oil

\* : after incubation, add the indicated reagent

## COLLECTION AND STORAGE OF THE SAMPLE

**ENTEROSYSTEM 18R** is used for the identification of Gram-negative, oxidase negative bacteria isolated on selective agar media for Enterobacteriaceae such as Mac Conkey Agar (ref. 10029), Eosin Methylene Blue Agar (ref. 10048), Salmonella and Shigella Agar (ref. 10036), Hektoen Enteric Agar (ref. 10043), or non-selective media.

## TEST PROCEDURE

### PREPARATION OF BACTERIAL SUSPENSION

1. The microorganism to be identified must be recently isolated (18-24 h); bacterial cultures older than 48 hours can provide not reliable results.
2. Before inoculating the microorganism to be examined, Gram staining and oxidase testing are required. Use **ENTEROSYSTEM 18R** with Gram-negative, oxidase negative bacteria only.
3. Take one or more morphologically similar well isolated colonies from the agar culture medium and suspend in physiological solution (0.5 McFarland bacterial suspension).
4. Thoroughly homogenize the suspension.

### INOCULATION OF THE SYSTEM

1. Take a system from its wrapper and bring it to room temperature.
2. Write down date and origin of the microorganism.
3. Transfer 0.2 mL of bacterial suspension into each well of the system and overlay with 1 drop of vaseline oil the wells **2-LDC, 3-ODC, 4-ADC, 7-UR** and **8-H<sub>2</sub>S**.
4. Cover the system with the lid provided and incubate at 36±1°C for 12-18-24 hours.

## INTERPRETATION OF THE RESULTS

At the end of the incubation period:

1. Add 2 drops of alpha-naphthol and 1 drop of NaOH 40% (ref. 80252) into the well **10-VP**. Wait for the development of a pink-red color in about 15-20 minutes.
2. Add 2 or 3 drops of KOVAC'S Reagent (ref. 80252) into the well **11-IND**. Wait for the development of a red color in about 1-2 minutes.
3. Watch for the color change in the wells and interpret the results using table 1.
4. Note the results on the test results form and determine the 6-digit code following instructions provided in the NUMERICAL CODE FORMATION paragraph.
5. Identify the organism by using the ENTEROSYSTEM 18R Code Book (ref. 71710) or the Identification Code Disk (ref. 71711).

Table 1.

Well	REACTIONS FOR THE BIOCHEMICAL IDENTIFICATION	Well color	
		Positive reaction	Negative reaction
1-ONPG	ONPG hydrolysis	yellow	colorless
2-LDC	Lysine decarboxylation	red	yellow-orange
3-ODC	Ornithine decarboxylation	red	yellow-orange
4-ADC	Arginine decarboxylation	red	yellow-orange
5-PD	Phenylalanine deamination	black-brown	yellow
6-CIT	Citrate utilization	blue-dark green	light green
7-UR	Urea hydrolysis	red-fuchsia	yellow-orange
8-H <sub>2</sub> S	Hydrogen sulphide production	black	yellow
9-MLN	Malonate utilization	blue-green	yellow
10-VP	VP test	pink-red	yellow
11-IND	Indole test	red	yellow
12-GLU	Glucose fermentation	yellow	blu-verde
13-MAN	Mannitol fermentation	yellow	blu-verde
14-INO	Inositol fermentation	yellow	blu-verde
15-SOR	Sorbitol fermentation	yellow	blu-verde
16-SAC	Saccharose fermentation	yellow	blu-verde
17-ARA	Arabinose fermentation	yellow	blu-verde
18-RAF	Raffinose fermentation	yellow	blu-verde

**NUMERICAL CODE FORMATION**

The biochemical tests are separated into 6 groups of 3 and a value of 1, 2 or 4 is indicated for each:

- Value 1 : first test positive in each group (**ONPG, ADC, UR, VP, MAN, SAC**);
- Value 2 : second test positive in each group (**LDC, PD, H<sub>2</sub>S, IND, INO, ARA**);
- Value 4 : third test positive in each group (**ODC, CIT, MLN, GLU, SOR, RAF**);
- Value 0 : every negative test.

A 6-digit code is obtained by adding together the values corresponding to positive reactions within each group. The code allows the identification of the organism under examination by using the ENTEROSYSTEM 18R Code Book (ref. 71710) or the Identification Code Disk software (ref. 71711). The example below shows how a numerical code can be formed.

**Example.**

	Group 1			Group 2			Group 3			Group 4			Group 5			Group 6		
Test	ONPG	LDC	ODC	ADC	PD	CIT	UR	H <sub>2</sub> S	MLN	VP	IND	GLU	MAN	INO	SOR	SAC	ARA	RAF
Values	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4
Results	+	+	+	-	-	-	-	-	-	-	+	+	+	-	+	-	+	-
Sum of values	7			0			0			6			5			2		
CODE: 700652 IDENTIFICATION: <i>Escherichia coli</i>																		

SIGNIFICANT BIOCHEMICAL REACTIONS FOR DIFFERENTIATING ENTEROBACTERIACEAE								
Microorganism	ONPG	UR	H <sub>2</sub> S	IND	VP	LDC	CIT	MLN
<i>Escherichia</i> spp	+	-	-	+	-	+	-	-
<i>Enterobacter</i> spp	±	±	-	-	+	±	+	+
<i>Klebsiella</i> spp	+	±	-	±	+	+	+	±
<i>Proteus</i> spp	-	+	±	±	-	-	±	-
<i>Salmonella</i> spp	-	-	±	-	-	±	-	-
<i>Citrobacter</i> spp	+	-	+	±	-	-	+	-
<i>Arizona</i> spp	±	-	+	±	-	+	±	±
<i>Yersinia</i> spp	±	±	-	±	±	-	-	±
<i>Serratia</i> spp	±	-	-	-	±	±	±	±

+ : positive reaction

- : negative reaction

± : variable reaction

### QUALITY CONTROL

**ENTEROSYSTEM 18R** is subjected to the quality control using the following reference microorganisms:

*Enterobacter cloacae* ATCC® 23355, *Escherichia coli* ATCC® 25922, *Klebsiella pneumoniae* ATCC® 13883, *Proteus mirabilis* ATCC® 25933, *Salmonella typhimurium* ATCC® 14028, *Serratia marcescens* ATCC® 8100, *Shigella flexneri* ATCC® 12022, *Yersinia enterocolitica* ATCC® 9610.

### FACTORS THAT MAY INVALIDATE THE RESULTS

Poor standardization of the inoculum; clinical material unsuitable; use of expired systems or expired supplementary reagents; non compliance with temperatures and times of incubation.

### PRECAUTIONS

The product, **ENTEROSYSTEM 18R** is not classified as hazardous under current legislation, however refer to the safety data sheet for a correct use. **ENTEROSYSTEM 18R** is a disposable device to be used only for diagnostic use *in vitro*. The product must be used in the laboratory by properly trained personnel, using approved aseptic and safety methods for handling pathogenic agents.

### STORAGE

Store the product **ENTEROSYSTEM 18R** at 2-8 °C in the original packaging. Keep away from sources of heat and avoid excessive changes in temperature. In such conditions the product will remain valid until the expiry date indicated on the label. Do not use beyond that date. Eliminate without using if there are signs of deterioration.








### DISPOSAL OF USED MATERIAL

After use, **ENTEROSYSTEM 18R** and material that has come into contact with the sample must be decontaminated and disposed of in accordance with the techniques used in the laboratory for decontamination and disposal of potentially infected material.

### PRESENTATION

Product	Ref.	Package
ENTEROSYSTEM 18R	71618	20 tests
ENTEROSYSTEM 18R	79618	4 tests

### TABLE OF SYMBOLS

<b>IVD</b> for <i>in vitro</i> diagnostic use	 Do not reuse	 Manufacturer	 Contains sufficient for <n> test	 Temperature limits
<b>REF</b> Catalogue number	 Fragile, handle with care	 Use by	 Caution, consult accompanying documents	<b>LOT</b> Batch number

