



EnteroPluri-Test

Identification system of *Enterobacteriaceae*
and other gram negative, oxidase negative bacteria.

DESCRIPTION

EnteroPluri-Test is a 12-sector system containing special culture media that permits identification of the *Enterobacteriaceae* and other gram negative, oxidase negative bacteria.

The system allows the simultaneous inoculation of all media present in the sectors and the execution of 15 biochemical reactions.

Microorganism is identified evaluating the colour change of the different culture media after 18-24 hours of incubation at 36 ± 1 °C and by a code number obtained from biochemical reaction interpretation.

CONTENT OF THE PACKAGE

Each package contains 25 **EnteroPluri-Test**.

ITEMS NECESSARY BUT NOT INCLUDED IN THE PACKAGE

Indole Reagent Droppers	BD Cat. No.: 261185
EnteroPluri-Test Codebook	BD Cat. No.: 215337
Oxidase Reagent Droppers	BD Cat. No.: 261181
Voges-Proskauer A and B Reagent Droppers	BD Cat. Nos.: 261192 and 261193

CONFIGURATION

The configuration of the system is shown in Table no. 1.

Table no. 1:

Sector	BIOCHEMICAL REACTIONS
Glucose/Gas	Glucose fermentation and gas production in anaerobiosis
Lysine	Lysine decarboxylation in anaerobiosis
Ornithine	Ornithine decarboxylation in anaerobiosis
H₂S/ Indole	Hydrogen sulphide production and indole test
Adonitol	Adonitol fermentation
Lactose	Lactose fermentation
Arabinose	Arabinose fermentation
Sorbitol	Sorbitol fermentation
VP	Acetoin production (Voges-Proskauer)
Dulcitol/PA	Dulcitol fermentation and phenylalanine deamination
Urea	Urea hydrolysis
Citrate	Citrate utilization

PRINCIPLE OF THE METHOD

EnteroPluri-Test makes possible the identification of the *Enterobacteriaceae* and other gram negative, oxidase negative bacteria isolated from non-clinical samples.

The identification is based on biochemical tests performed on culture media containing specific substrates. The combination of positive and negative reactions allows to build up a code number that permits to identify bacteria by using the **Codebook**.

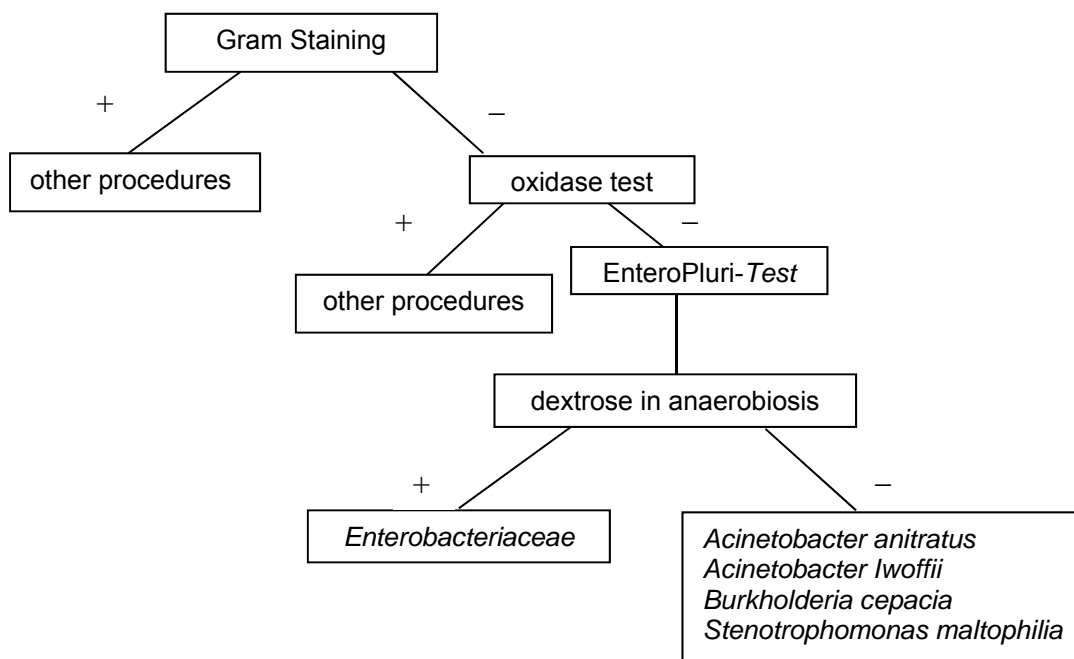
SAMPLE COLLECTION

EnteroPluri-Test is used for identification of gram negative, oxidase negative bacteria isolated in selective culture media for *Enterobacteriaceae* growth as: MacConkey Agar (MCA), Eosin Methylene Blue Agar (EMBA), Salmonella and Shigella Agar (SSA), Hektoen Enteric Agar (HEEA), or in not selective culture media.

TEST PROCEDURE

The microorganism to be identified should be recently isolated (18-24 hours): bacteria from cultures older than 48 hours can provide unreliable results.

Before inoculating the microorganism to be identified, it's compulsory to perform a gram staining and oxidase test on the microorganism. Only gram negative, oxidase negative bacteria should be inoculated on **EnteroPluri-Test**. For the correct performance of both tests please consult appropriate bacteriology manuals.



- Pick up an **EnteroPluri-Test** system from the package and note identificative name of bacterial strain to submit to identification, date of test and other useful information.
- Remove both caps of the system. Using the tip of inoculating needle, placed under the white cap, and without flaming, pick up a well isolated colony from a selective or non selective agar medium, without penetrating into the agar.
- Inoculate **EnteroPluri-Test** turning and withdrawing the needle throughout the sectors of the system.

- Reinsert the needle with a turning movement until the breakage notch; break the inoculating needle folding it in correspondence with the notch. The portion of the needle remaining inside the system keeps anaerobic conditions necessary for reactions of the sectors **Glucose/Gas**, **Lysine** and **Ornithine**.
- Use the broken portion of the needle, remained in the user hands, to punch the plastic film in correspondence of the holes of the sectors **Adonitol**, **Lactose**, **Arabinose**, **Sorbitol**, **VP**, **Dulcitol/PA**, **Urea**, **Citrate** in order to support aerobic growth.
- Screw again both caps and incubate **Enteropluri-Test** at 36 ± 1 °C for 18-24 hours, putting it on its flat surface or vertically in a test-tube holder with the sector Glucose/Gas pointing upward.

INTERPRETATION OF RESULTS

At the end of incubation:

- Observe the change in colour of culture media in the different sectors and interpret results using the table n° 2 and, in case, an **Enteropluri-Test** not inoculated and kept at room temperature.

NOTE: if there is no change in colour in the sector **Glucose/Gas** while in some other sectors there are chromatic changes, the microorganism under test does not belong to the family of *Enterobacteriaceae*. The **Codebook** also includes many codes of microorganisms that do not ferment glucose in anaerobiosis, but sometimes some additional biochemical reactions may be necessary for a correct identification of these nonfermenters.

- Record the obtained results on the enclosed data chart, except Indole test (sector **H₂S/Indole**) and Voges-Proskauer test (sector **VP**). Then perform Indole and Voges-Proskauer tests.

Indole test

Lay **Enteropluri-Test** with its flat surface pointing upward and, by punching the plastic film, add 3 or 4 drops of Kovac's Indole Reagent in the sector **H₂S/Indole**.

The reaction is positive if a pink-red colour develops in the added reagent within 10-15 seconds.

Voges-Proskauer test

This test is required if the database with VP is used; if the database without VP is used, it may be run as a confirmatory test.

Lay **Enteropluri-Test** with its flat surface pointing upward and, by punching the plastic film, add 3 drops of α -naphthol and 2 drops of potassium hydroxide.

The reaction is positive if a red colour develops within 20 minutes.

- Form the 5-digit code following the instructions provided in the paragraph **CODE NUMBER FORMING**. Identify the bacterium using the **Codebook**.

Table no. 2:

Sector	BIOCHEMICAL REACTIONS	Sector colour	
		Positive reaction	Negative reaction
Glucose / Gas	Glucose fermentation	yellow	red
	Gas production	lifted wax	overlying wax
Lysine	Lysine decarboxylation	violet	yellow
Ornithine	Ornithine decarboxylation	violet	yellow
H₂S / Indole	Hydrogen sulphide production	black-brown	beige
	Indole test	pink-red	colourless
Adonitol	Adonitol fermentation	yellow	red
Lactose	Lactose fermentation	yellow	red
Arabinose	Arabinose fermentation	yellow	red
Sorbitol	Sorbitol fermentation	yellow	red
VP	Acetoin production	red	colourless
Dulcitol/PA	Dulcitol fermentation	yellow	green
	Phenylalanine deamination	dark brown	green
Urea	Urea hydrolysis	purple	beige
Citrate	Citrate utilisation	blue	green

CODE NUMBER FORMING: DATABASE WITH VP

1) The 15 biochemical tests are divided into 5 groups each containing 3 tests and each test is indicated with a positivity value of 4, 2, 1.

- Value 4 : first test positive in each group (**Glucose, Ornithine, Adonitol, Sorbitol, PA**)
- Value 2 : second test positive in each group (**Gas, H₂S, Lactose, VP, Urea**)
- Value 1 : third test positive in each group (**Lysine, Indole, Arabinose, Dulcitol, Citrate**)
- Value 0 : every negative test

2) Adding the number of positive reactions in each group, it is obtainable a 5 digit code which, by the use of the **Codebook**, allows the identification of the microorganism under examination as in the following example.

Test	Group 1			Group 2			Group 3			Group 4			Group 5		
	Glucose	Gas	Lysine	Ornithine	H ₂ S	Indole	Adonitol	Lactose	Arabinose	Sorbitol	VP	Dulcitol	PA	Urea	Citrate
Positivity code	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1
Results	+	+	-	+	+	-	-	-	-	-	+	-	+	+	-
Code	4+2+0=6			4+2+0=6			0+0+0=0			0+2+0=2			4+2+0=6		
CODE: 66026 IDENTIFICATION: <i>Proteus mirabilis</i>															

The code obtained in the above example is located in section ENTEROBACTERIACEAE. At the number read as follows:

ENTEROBACTERIACEAE (metodo con VP)		
ENTEROBACTERIACEAE (method with VP)		
Codice numerico Code number	Microrganismo Microorganism	Test atipici Atypical Tests
66026	<i>Proteus mirabilis</i>	NONE

CONCLUSION: the code 66026 identifies the microorganism *Proteus mirabilis*. No atypical test found. Confirmatory tests are not necessary.

CODE NUMBER FORMING: DATABASE WITHOUT VP

1) The 14 biochemical tests are divided into 5 groups; Group 1 consists of 2 tests, while Groups 2-5 consist of 3 tests. Each test is indicated with a value of positivity of 4,2,1.

- Value 4 : first test positive in groups 2,3,4 and 5 (**Lysine, Indole, Arabinose, PA**)
- Value 2 : first test positive in first group and second test positive in remaining groups (**Glucose, Ornithine, Adonitol, Sorbitol, Urea**)
- Value 1 : second test positive in first group and third test positive in remaining groups (**Gas, H₂S, Lactose, Dulcitol, Citrate**)
- Value 0 : every negative test

2) Adding the number of positive reactions in each group, it is obtainable a 5 digit code which, by the use of the **Codebook** (Database without VP), allows the identification of the microorganism under examination as in the following examples.

An asterisk (*) next to the identification indicates the presence of a rare organism. If a rare organism is the best choice, check the purity of the isolate and repeat the inoculation. Also, if a rare organism is encountered as the first choice, and common microbes are also listed, confirmatory tests must be done. The frequency of occurrence of these rare isolates is so low (often less than once per year) that a technical error such as a mixed or light inoculum should be ruled out before reporting the results.

EXAMPLE 1

Test	Group 1		Group 2			Group 3			Group 4			Group 5			
	Glucose	Gas	Lysine	Ornithine	H ₂ S	Indole	Adonitol	Lactose	Arabinose	Sorbitol		Dulcitol	PA	Urea	Citrate
Positivity code	2	1	4	2	1	4	2	1	4	2		1	4	2	1
Results	+	+	+	+	-	-	+	-	+	+		-	-	-	+
Code	2+1=3		4+2+0=6			0+2+0=2			4+2+0=6			0+0+1=1			
CODE: 36261 IDENTIFICATION: <i>Enterobacter aerogenes</i> – <i>Serratia liquefaciens</i>															

The code 36261 obtained in the above example is located in section ENTEROBACTERIACEAE. At the number read as follows:

ENTEROBACTERIACEAE (metodo senza VP)		
ENTEROBACTERIACEAE (method without VP)		
Codice numerico Code number	Microrganismo Microorganism	Test atipici Atypical Tests
36261	<i>Enterobacter aerogenes</i>	LAC -
	<i>Serratia liquefaciens</i>	ADO +

CONCLUSION: the code 36261 identifies the microorganism *Enterobacter aerogenes* or *Serratia liquefaciens*. Confirmatory test should be carried out to obtain an accurate identification. For each microorganism are indicated the corresponding atypical tests. Any biochemical test result, obtained with **EnteroPluri-Test**, which is improbable for a given species, when compared with the **Table of biochemical reactions** (Table no. 4), is considered an atypical test.

EXAMPLE 2

Test	Group 1		Group 2			Group 3			Group 4			Group 5			
	Glucose	Gas	Lysine	Ornithine	H ₂ S	Indole	Adonitol	Lactose	Arabinose	Sorbitol		Dulcitol	PA	Urea	Citrate
Positivity code	2	1	4	2	1	4	2	1	4	2		1	4	2	1
Results	+	+	+	+	-	-	-	-	+	+		-	-	-	+
Code	2+1=3		4+2+0=6			0+0+0=0			4+2+0=6			0+0+1=1			
CODE: 36061 IDENTIFICATION: <i>Serratia liquefaciens</i>															

The code 36061 obtained in the above example is located in section ENTEROBACTERIACEAE. At the number read as follows:

ENTEROBACTERIACEAE (metodo senza VP)		
ENTEROBACTERIACEAE (method without VP)		
Codice numerico Code number	Microorganismo Microorganism	Test atipici Atypical Tests
36061	<i>Serratia liquefaciens</i>	NONE

CONCLUSION: the code 36061 identifies the microorganism *Serratia liquefaciens*. No atypical test found. Confirmatory tests are not necessary.

USER QUALITY CONTROL

Inoculate **EnteroPluri-Test** using the reference bacterial strains indicated in the table no. 3.

For inoculation, incubation and reading please follow the instructions indicated in the paragraph **TEST PROCEDURE**.

Table no. 3:

Microorganisms	Glucose	Gas	Lysine	Ornithine	H ₂ S	Indole	Adonitol	Lactose	Arabinose	Sorbitol	VP	Dulcitol	PA	Urea	Citrate	Acceptable biocodes
<i>Escherichia coli</i> ATCC 25922	+	±	+	+	-	+	-	+	+	+	-	-	-	-	-	75340
<i>Proteus mirabilis</i> ATCC 25933	+	±	-	+	+	-	-	-	-	-	±	-	+	+	±	66007
<i>Klebsiella pneumoniae</i> ATCC 13883	+	±	+	-	-	-	+	+	+	+	±	+	-	±	+	70773-70771 70753-70751
<i>Salmonella typhimurium</i> ATCC 14028	+	±	+	±	+	-	-	-	+	+	-	-	-	-	±	52140
<i>Pseudomonas aeruginosa</i> * ATCC 27853	-	-	-	-	-	-	-	-	±	-	-	-	-	±	+	*

Pseudomonas aeruginosa* is oxidase positive, therefore it is not included in the **EnteroPluri-Test Codebook.

TABLE OF BIOCHEMICAL REACTIONS

Table no. 4: Percentage of strains giving positive reactions with 18-24 h incubation at 36 ± 1 °C

		Glucose	Gas	Lysine	Ornithine	H ₂ S	Indole	Adonitol	Lactose	Arabinose	Sorbitol	Voges-Proskauer	Dulcitol	Phenylalanine	Urea	Citrate	
Escherichieae	<i>Escherichia</i>	+	+J	d	d	-K	+	-	+J	+	+/-	-	d	-	-	-	
	<i>Shigella</i>	+	-A	-	-/+B	-	-/+	-	-B	+/-	-/+	-	d	-	-	-	
Edwardsiellae	<i>Edwardsiella</i>	+	+	+	+	+	+	-	-	+/-	-	-	-	-	-	-	
Salmonelleae	<i>Salmonella</i>	+	+C	+H	+I	+E	-	-	-	+/-	+	-	dD	-	-	dF	
	<i>Arizona</i>	+	+	+	+	+	-	-	D	+	+	-	-	-	-	+	
	<i>freundii</i>	+	+	-	d	+/-	-	-	d	+	+	-	d	-	dw	+	
	<i>amalonaticus</i>	+	+	0.0	+	0.0	+	+	+/-	+	+	0.0	-/+	0.0	+/-	+	
	<i>diversus</i>	+	+	0.0	+	0.0	+	+	d	+	+	-	+/-	-	dw	+	
	<i>Proteus</i>	<i>vulgaris</i>	+	+/-G	-	-	+	+	-	-	-	-	-	-	+	+	d
<i>mirabilis</i>	+	+G	-	+	+	-	-	-	-	-	-/+	-	+	+/-	+/-		
<i>Morganella</i>	<i>morganii</i>	+	+/-G	-	+	+	-	-	-	-	-	-	+	+	-		
<i>Providencia</i>	<i>alcalifaciens</i>	+	dG	0.0	-	-	+	+	-	-	-	0.0	0.0	+	-	+	
	<i>stuartii</i>	+	-	0.0	0.0	0.0	+	-/+	-	-	-	-	+	-/+	+		
	<i>rettgeri</i>	+	-/+G	-	-	-	+	+	d	-	-	-	+	+	+		
Klebsielleae	<i>Enterobacter</i>	<i>cloacae</i>	+	+	-	+	-	-	-/+	+/-	+	+	d	-	-/+	+	
	<i>sakazakii</i>	+	+	0.0	+	0.0	-/+	-	+	+	+	-	-	-	-	+	
	<i>gergoviae</i>	+	+	+/-	+	-	-	-	-/+	+	-	+	-	-	+	+	
	<i>aerogenes</i>	+	+	+	+	-	-	+	+	+	+	100.0	4.1	0.0	0.0	+	
	<i>Pantoea</i>	<i>agglomerans</i>	+	-/+	-	-	-	-/+	-	d	+	d	+/-	d	-/+	d	
	<i>Hafnia</i>	<i>alvei</i>	+	+	+	+	0.0	0.0	-	d	+	+	+/-	2.4	0.0	-	d
	<i>Serratia</i>	<i>marcescens</i>	+	+/-G	+	+	-	-W	-/+	-	-	+	+	-	-	dw	+
		<i>liquefaciens</i>	+	d	+/-	+	-	-W	-	d	+	+	+/-	-	-	dw	+
		<i>rubidaea</i>	+	dG	+/-	-	-	-W	+/-	+	+	+	+	-	-	dw	+/-
	<i>Klebsiella</i>	<i>pneumoniae</i>	+	+	+	-	-	-	+/-	+	+	+	+	-/+	-	+	+
<i>oxytoca</i>		+	+	+	-	-	+	+/-	+	+	+	+	-/+	-	+	+	
<i>ozaenae</i>		+	d	-/+	-	-	-	+	d	+	+/-	-	-	-	d	d	
<i>rhinoscleromatis</i>		+	-	-	-	-	-	+	d	+	+	-	-	-	-	-	
Yersineae	<i>Yersinia</i>	<i>enterocolitica</i>	+	0.0	0.0	+	-	-/+	-	+	+	-	0.0	0.0	0.0	0.0	
	<i>pseudotuberculosis</i>	+	-	-	-	-	-	-	-	+/-	-	-	-	-	+	-	

+ Positive
 - Negative
 +/- Mostly positive
 -/+ Mostly negative
 d Different biochemical types
 w Weak reaction
 A Certain biotypes of *S.flexneri* form gas
 B *S.sonnei* strain usually ferment lactose very slowly
 C *S.typhi* and *S.gallinarum* are anaerogenic
 D *S.typhi*, *S. cholerae-suis*, *S.enteritidis* bioserotypes *paratyphi* A and *pullorum*, and a few others do not ferment dulcitol promptly
 E *S.enteritidis* bioserotypes *paratyphi* A and some rare biotypes may be H₂S-negative
 F *S.typhi*, *S.enteritidis* bioserotypes *paratyphi* A and some rare biotypes are citrate-negative. *S.cholerae-suis* is usually delayed positive
 G *Serratia*, *Proteus* and *Providencia alcalifaciens* develop a little quantity of gas. Their gas production may be not evident
 H *S.enteritidis* bioserotype *paratyphi* A is lysine-negative
 I *S.typhi* and *S.gallinarum* are ornithine-negative
 J The *Alkalescens-Dispar* (A-D) group is included as a biotype of *E.coli*. Members of the A-D group are generally nonmotile, lactose-negative and do not form gas
 K An occasional strain may produce hydrogen sulfide

FACTORS THAT MAY INVALIDATE THE RESULTS

- Use of mixed cultures.
- Application of the method to bacteria not belonging to the family of *Enterobacteriaceae* or to non gram negative, oxidase negative bacteria.
- Use of expired systems.
- Test procedure different from the one suggested.

PRECAUTION

The product, **EnteroPluri-Test**, cannot be classified as hazardous under current legislation and does not contain harmful substances in concentrations $\geq 1\%$. It therefore does not require a Safety Data Sheet to be available. **EnteroPluri-Test** is a disposable device to be used only for identification from non-clinical samples; it is intended for use in a professional environment and should be used in laboratory by properly trained personnel, using approved asepsis and safety methods for handling pathogenic agents.

STORAGE

Store at 2-8 °C away from light. 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 them if there are signs of deterioration.

DISPOSAL OF USED MATERIAL

After use, **EnteroPluri-Test** should be decontaminated and disposed of in accordance with the techniques used in the laboratory for decontamination and disposal of potentially infected material.











BIBLIOGRAPHY

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PRESENTATION

Product	BD Cat. No.	Package
EnteroPluri-Test	215336	25 test

TABLE OF SYMBOLS

 Catalogue number	 Do not reuse	 Manufacturer	 Contains sufficient for <n> tests	 Temperature limitation
 Store away from light	 Fragile, handle with care	 Use by	 Caution, consult accompanying documents	 Batch code

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