RapiD 20 E

For in vitro diagnostic use

System for the identification of *Enterobacteriaceae* in 4 hours

RapiD 20 E is a system which enables the identification of *Enterobacteriaceae* in only 4 hours after the microorganism has been isolated. This standardized system combines 20 biochemical tests chosen for their highly discriminant value and adapted to rapid interpretation. The complete list of those organisms that it is possible to identify with this system is given in the Identification Table at the end of this package insert.

PRINCIPLE

The RapiD 20 E strip consists of 20 microtubes containing dehydrated substrates for the demonstration of enzymatic activity or the fermentation of sugars. A bacterial suspension distributed into the tubes dissolves the substrates. During the 4-hour incubation period, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the Interpretation of Reactions Table 2 and the identification is obtained by referring to the Analytical Profile Index or using the identification software.

REAGENTS

Kit contents (25 tests) :

- 25 RapiD 20 E strips
- 25 incubation boxes
- 25 polyethylene pipettes
- 25 result sheets
- 1 package insert

Additional products (not included in the kit) :

- NaCl 0.85 % Medium (Prod. No. 20710)
- Reagents : Potassium hydroxide (Prod. No. V7053)
  VP2 **or** (Prod. No. 70430)
  α-naphthol (Prod. No. V7054)
  Kovacs' Reagent (Prod. No. V7050)
  Oxidase Test Kit (Prod. No. V7062)
- Mineral oil (Prod. No. 70100)
- McFarland Standard (Prod. No. 70900)
- RapiD 20 E Analytical Profile Index (Prod. No. 20790 or identification software (consult bioMérieux)
- Ampule stand (Prod. No. 70200)

Required laboratory equipment :

- 35-37°C incubator
- Refrigerator
- Bunsen burner
- Marker pen

COMPOSITION OF MEDIA AND REAGENTS

NaCl 0.85 % Medium 1.25 ml	Sodium chloride 8.5 g Demineralized water to make 1000 ml
Potassium hydroxide 30 ml	40% aqueous KOH solution 89% in demineralized water 11% **CORROSIVE** R35 : Causes severe burns. S24/25: Avoid contact with skin and eyes. S26 : In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36/37/39 : Wear suitable protective clothing,gloves and eye/face protection. S45: In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).
Voges-Proskauer reagent : **VP2** 5 ml **or** **α-naphthol** 30 ml	Alpha-naphthol 6 g Ethyl alcohol 100 ml Alpha-naphthol 2.05 g to be diluted with 95% ethyl alchol 29 ml **FLAMMABLE AFTER RECONSTITUTION** R21/22: Harmful in contact with skin and if swallowed. S24/25: Avoid contact with skin and eyes.
Kovacs' Reagent 30 ml	N-amyl alcohol 71% Paradimethylaminobenzaldehyde 5% Concentrated hydrochloric acid 24% **HARMFUL** R10:Flammable. R20:Harmful by inhalation. R34: Causes burns. R37: Irritating to respiratory system. S24/25 : Avoid contact with skin and eyes. S16: Keep away from sources of ignition. No smoking. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36/37/39: Wear suitable protective clothing, gloves and eye/face protection. S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
Oxidase Test Kit	Oxidase Reagent: see Oxidase Test Kit Package Insert.

STORAGE OF THE STRIPS AND MEDIA

The strips and media should be stored at 2-8°C until the expiration date indicated on the packaging.

STORAGE OF THE REAGENTS

Potassium hydroxide and Kovacs' Reagent should be stored at 15-30°C until the expiration date indicated on the packaging. After reconstitution, α-naphthol should be stored at 2-8°C and may be kept for up to 90 days, (or until the expiration date if this comes first) : record the date opened on the bottle label.
VP2 should be stored at 2-8°C. until the expiration date if this comes first. VP2 may be kept for up to 1 month after the ampules have been opened and the reagent transferred to the dropper-bottle.

USE OF THE REAGENTS

Allow reagents to come to room temperature (20-30°C) before using.

1. Potassium hydroxide, VP2 or α-naphthol, and Kovacs' reagents:

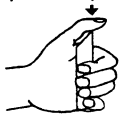
- Open the ampule of reagent as indicated in the paragraph "Warnings andPrecautions" (ampule with dropper-cap).
- Dispense one drop of reagent.
- Carefully close the bottle after use and store it as indicated in the paragraph "Storage of the reagents".

2. Oxidase Reagent:

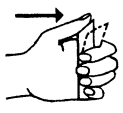
- Please note that the oxidase test must be performed as it is an integral part of the final profile (21st identification test).
- See Oxidase Test Kit Package Insert for the use of the oxidase reagent.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Qualified laboratory personnel should use aseptic technique and established precautions for infectious agents.
- Do not pipette specimens or reagents by mouth.
- Do not use reagents past the expiration date.
- Do not allow reagents to come in contact with skin, eyes or clothing.
- Upon removal from refrigerator, allow reagents to come to room temperature (20-30°C) before using.
- Open ampules carefully as follows :



- Hold the ampule in one hand in a vertical position (white plastic cap uppermost).
- Press the cap down as far as possible.
- Cover the flattened part of the cap with the upper part of the thumb.



- Apply thumb pressure in an outward motion to the flattened part of the cap to snap off the top of the ampule inside the cap.
- Carefully remove the cap.

** For ampule with no dropper-cap :*
- Carefully remove the cap.

** For ampule with dropper-cap :*
- Turn the ampule upside down and maintain it in a vertical position.
- Squeeze on the cap to transfer all the reagent into the dropper-bottle.

- All inoculated products should be considered infectious and handled appropriately.
- All patient specimens and microbial cultures are potentially infectious and should be treated with universal precautions (NCCLS M29-A: *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*: Approved Guideline 1997).
- After completing test, reading and interpretation, all specimens, spills and inoculated products must be autoclaved, incinerated or immersed in a germicide prior to disposal.
- Interpretation of the test results should be made by a competent microbiologist who should also take into consideration the patient history, the source of the specimen, colonial and microscopic morphology and, if necessary, the results of any other tests performed, particularly the antimicrobial susceptibility patterns.
- The performance data presented were obtained using the procedures indicated. Any changes or modifications in the procedures may affect results.

INSTRUCTIONS FOR USE

Specimens and bacterial cultures should be considered infectious and handled appropriately by trained and competent technicians.
Aseptic technique and usual handling precautions for the bacterial group studied should be observed throughout this procedure ; refer to Universal Precautions (NCCLS M29-A, *Protection of Laboratory Workers from Instrument Biohazards and Infectious Diseases Transmitted by Blood, Body Fluids, and Tissue*: Approved Guideline 1997).
For additional handling precautions, refer to Biosafety in Microbiological and Biomedical Laboratories, HHS Publication No. (CDC) 93-8395, 3rd Edition (May 1993), or to the regulations of each country.

Selection of colonies

RapiD 20 E is to be used exclusively for members of the *Enterobacteriaceae*. In general, only oxidase-negative and Gram-negative bacilli should be tested with this strip.

It is therefore necessary to pick a few morphologically identical colonies on the isolation plate and to perform an oxidase test on one of them. Refer to the Oxidase Test Kit Package Insert.

This reaction should be recorded on the result sheet as it constitutes the 21st test.

NOTE : Certain Gram-negative bacilli not belonging to the *Enterobacteriaceae* and oxidase-positive (*Aeromonas* and *Vibrio*) are perfectly identifiable by RapiD 20 E. Clinical or bacteriological features will dictate whether to use this strip.

Preparation of the strip

- Prepare an incubation box (tray and lid). It is not necessary to add water to the tray.
- Record the strain reference on the elongated flap of the tray.
- Remove the strip from its packaging, place it in the tray and discard the desiccant.

Preparation of the inoculum

- Open a scored ampule of NaCl 0.85 % Medium as indicated in the paragraph "Warnings and Precautions" or use any tube containing 1.25 ml of 0.85 % physiological saline without additives.
- Using a pipette included in the kit, pick 1-4 colonies from the agar plate either by suction or by successive touches. **Only young cultures should be used (18-24 hours old).**
- Make a bacterial suspension with a turbidity equal to 0.5 McFarland.
- With this suspension, streak a purity plate.

NOTE : In order to facilitate their use, the polyethylene pipettes, protected by their sheaths, should be placed in a receptacle, point downwards.

Inoculation of the strip

- With the same pipette, distribute the suspension into the tubes of the strip. Avoid the formation of bubbles at the base of the tubes by placing the tip of the pipette on the side of the cupule and tilting the strip slightly forwards.
  - For the CIT test, add 2 drops of the suspension (approx. 50 µl) to fill the tube and lower portion of the cupule.
  - For the other tests, only fill the tubes (approx. 50 µl per tube). **The accuracy of the filling is very important.** Tubes insufficiently filled or overfilled may be the source of false positive and false negative results.
  - For the underlined tests (LDC, ODC and URE), completely fill the cupule with mineral oil.
  - Close the incubation box and incubate at 35-37°C for 4 hours.

Reading the strip

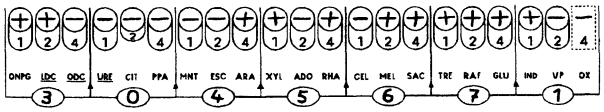
- After the incubation period, read the strip by referring to the Interpretation of Reactions (Table 2).
- Record all spontaneous reactions on the result sheet. For the fermentation tests (sugar substrates), a **green** color indicates the onset of acidification and should be considered as a **positive** reaction.
- Reveal the VP and IND tests by adding the corresponding reagents :
  - VP Test : add 1 drop of each of Potassium hydroxide and VP2 **or** α-naphthol reagents. Wait 5-10 minutes. A **red** color indicates a **positive** reaction to be recorded on the result sheet.
  - IND Test : add 1 drop of Kovacs' reagent. Read within 3 minutes. A **red ring** indicates a **positive** reaction to be recorded on the result sheet.

NOTE : Do not put the lids back on the strips during the reading of the results.

Identification

Identification can be obtained:

- using the Analytical Profile Index : the pattern of the reactions obtained must be coded into a numerical profile. On the result sheet, the tests are separated into groups of 3 and a number 1, 2 or 4 is indicated for each. By adding together the numbers corresponding to positive reactions within each group, a 7-digit profile number is obtained. The oxidase reaction constitutes the 21st test and has a value of 4 if it is positive.
- To obtain information on any profile not listed in the Analytical Profile Index, call the Voice Response System at 800-645-7056.
- using the identification software .



3 045 671 *Escherichia coli*

DISPOSAL OF USED MATERIAL

After use, all ampules, pipettes, strips and incubation boxes should be autoclaved, incinerated, or immersed in a disinfectant for decontamination prior to disposal.

LIMITATIONS

- The RapiD 20 E strip is intended for the identification of *Enterobactericeae*. A few other oxidase-positive and Gram-negative bacilli displaying both fermentative and oxidative metabolism (*Aeromonas, Vibrio*) give interpretable results. In general, only oxidase-negative and Gram-negative bacilli should be tested with this strip.
- The biochemical identification of *Salmonella*, *Shigella* as well as enteropathogenic *Escherichia coli* should be considered as presumptive and be confirmed by serology.
- The RapiD 20 E strip is intended for the identification of *Enterobactericeae* in 4 hours. Nevertheless, the strip may be read after 5 hours of incubation. Under no circumstances should this 5-hour limit be exceeded as there exists a possibility of erroneous identification.
- In order for the RapiD 20 E tests to function properly, it is imperative that the inoculum density be adjusted to 0.5 McFarland. In particular, a weaker inoculum may lead to false negative results.

RANGE OF EXPECTED VALUES

The RapiD 20E tests produce color reactions (interpreted as positive or negative) which make identification possible. Absolute quantitative values are not obtained.

PERFORMANCE CHARACTERISTICS

The RapiD 20E system has been evaluated by Flandrois (4) and Thomas (9). These studies have shown that the RapiD 20E system identifies *Enterobacteriaceae* as accurately as conventionally accepted methods. For specific performance characteristics, see enclosed charts.

QUALITY CONTROL

The media, strips, and reagents are systematically quality controlled at various stages of their manufacture. For those who wish to perform their own quality control tests with the strip, it is recommended that the following stock cultures be used, to obtain the results below :

Table 1

	ONPG	LDC	ODC	URE	CIT	PPA	MNT	ESC	ARA	XYL	ADO	RHA	CEL	MEL	SAC	TRE	RAF	GLU	IND	VP
1.	+	+	+	–	–	–	–	–	+	–	–	+/–	–	+	–	+	–	+	+	–
2.	–	–	–	+	–	+	–	–	–	+/–	–	–	–	–	+/–	–	–	+/–	+	–
3.	+	+	–	V	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+*

* Occasional weak reactions may occur.

1. *Escherichia coli* ATCC 11775
2. *Proteus vulgaris* ATCC 13315
3. *Klebsiella pneumoniae* ssp *pneumoniae* ATCC 35657

ATCC : American Type Culture Collection,
10801 University Boulevard, Manassas, VA 20110-2209, USA

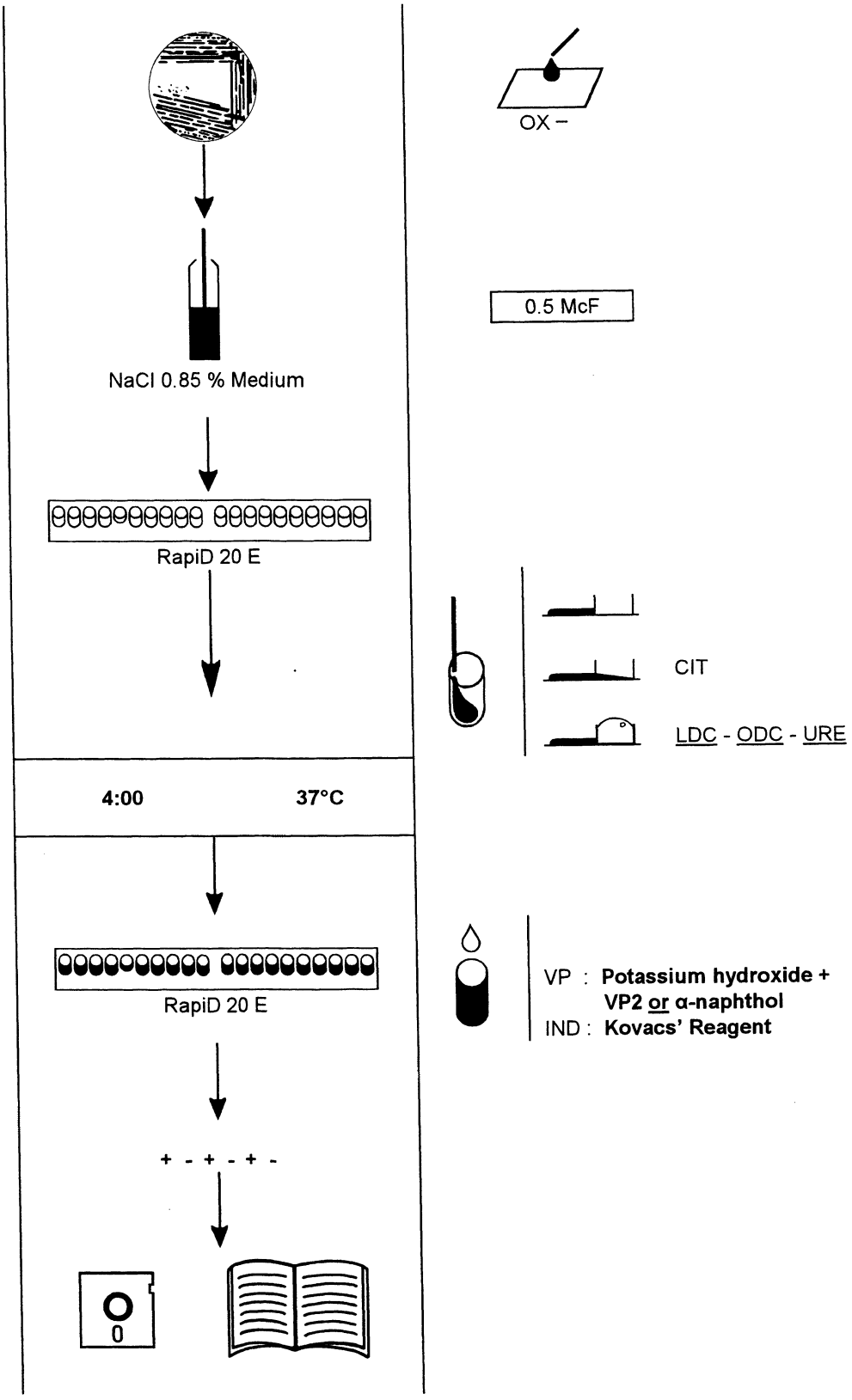
INTERPRETATION OF REACTIONS

Table 2

TESTS	SUBSTRATES	QTY	REACTIONS/ENZYMES	RESULTS	
				NEGATIVE	POSITIVE
ONPG	o-nitrophenyl-β-D-galactopyranoside(ONPG) isopropylthiogalacto-pyranoside (IPTG)	95.0µg 3.0µg	β-D-galactosidase	colorless	pale yellow to bright yellow
LDC	lysine	0.65 mg	lysine decarboxylase	yellow-green to bluish-grey	blue to blue-violet
ODC	ornithine	0.4 mg	ornithine decarboxylase	yellow-green to bluish-grey	blue to blue-violet
URE	urea	0.4 mg	urease	yellow	pink / pink-violet
CIT	sodium citrate	0.16 mg	utilization	yellow to yellow-green	green to blue
PPA	phenylalanine	30.0 µg	phenylalanine deaminase	colorless	orange-brown
MNT	malonate	0.12 mg	utilization	yellow	green to blue
ESC	esculin	0.1 mg	β-glucosidase	colorless	grey to black
ARA	arabinose	0.8 mg	acidification	blue	green to yellow
XYL	xylose	0.8 mg	acidification	blue	green to yellow
ADO	adonitol	0.8 mg	acidification	blue	green to yellow
RHA	rhamnose	0.8 mg	acidification	blue	green to yellow
CEL	cellobiose	0.8 mg	acidification	blue	green to yellow
MEL	melibiose	0.8 mg	acidification	blue	green to yellow
SAC	sucrose	0.8 mg	acidification	blue	green to yellow
TRE	trehalose	0.8 mg	acidification	blue	green to yellow
RAF	raffinose	0.8 mg	acidification	blue	green to yellow
GLU	glucose	0.8 mg	acidification	blue	green to yellow
IND	tryptophane	0.15 mg	indole formation	Kovacs' (1 drop) / 3 min yellow ring	red ring
VP	pyruvate creatine	0.1 mg 65.0 µg	acetoin production	Potassium hydroxide (1 drop) + VP2 or α-naphthol (1 drop) / 5-10 min colorless	red

NOTE: An oxidase test is required. See the INTERPRETATION OF REACTIONS in the Oxidase Test Kit Package Insert

RECOMMENDED METHODOLOGY



IDENTIFICATION TABLE

% of positive reactions after 4 hr. at 35-37°C

RAPID 20 E V3.0	ONPG	LDC	ODC	URE	CIT	PPA	MNT	ESC	ARA	XYL	ADO	RHA	CEL	MEL	SAC	TRE	RAF	GLU	IND	VP	OX
Buttiauxella agrestis	100	0	64	0	66	0	89	99	97	89	0	89	100	89	0	97	75	100	0	0	0
Cedecea spp	69	0	34	0	83	0	73	38	1	26	0	0	76	0	65	96	0	99	0	34	0
Citrobacter amalonaticus/farmeri	89	0	92	1	60	0	1	70	97	91	0	99	99	5	4	99	1	100	99	0	0
Citrobacter koseri	96	0	99	0	99	0	75	53	96	94	99	99	94	0	38	99	0	100	99	0	0
Citrobacter freundii group	86	0	30	0	50	0	10	4	92	94	1	88	24	62	60	97	46	100	1	0	0
Edwardsiella hoshinae	0	100	100	0	3	0	33	0	3	0	0	0	0	0	99	100	0	100	85	0	0
Edwardsiella tarda	0	100	100	0	1	0	0	0	0	0	0	0	0	0	0	0	0	100	99	0	0
Enterobacter aerogenes	100	99	99	1	95	0	90	99	99	99	99	98	99	85	99	99	98	100	0	94	0
Enterobacter asburiae	100	0	92	0	13	0	0	96	100	100	0	0	98	0	100	100	66	100	0	13	0
Enterobacter cloacae	99	2	87	1	95	0	71	51	99	84	25	81	98	79	88	99	81	100	1	81	0
Enterobacter gergoviae	90	85	100	80	68	0	99	99	99	75	2	97	1	90	99	85	75	100	0	75	0
Enterobacter cancerogenus	100	1	55	1	97	0	99	70	100	98	0	98	55	1	5	100	1	100	0	96	0
Escherichia coli 1	96	82	57	3	0	0	1	2	79	66	7	70	2	59	30	76	23	100	91	0	0
Escherichia coli 2	10	41	22	2	2	0	1	1	70	70	8	33	1	14	5	88	8	100	96	0	0
Escherichia fergusonii	87	95	96	0	0	0	0	0	52	87	57	61	87	0	0	74	0	100	100	0	0
Escherichia hermannii	75	0	100	0	25	0	0	14	100	87	0	87	99	0	1	99	0	100	100	0	0
Escherichia vulneris	100	74	33	0	8	0	74	11	91	82	16	16	83	83	0	99	83	100	0	0	0
Ewingella americana	90	0	0	0	95	0	0	40	0	1	0	1	10	0	0	100	0	100	0	80	0
Hafnia alvei	25	98	93	1	5	0	35	24	46	26	0	19	1	1	5	95	1	100	0	15	0
Klebsiella ornithinolytica	100	88	100	45	100	0	100	100	100	100	88	99	91	100	100	100	100	100	100	66	0
Klebsiella oxytoca	100	98	1	58	73	0	46	99	99	95	93	97	100	99	100	98	100	100	100	94	0
Klebsiella pneumoniae ssp ozaenae	95	23	1	1	65	0	3	88	67	62	95	35	55	75	10	88	68	99	0	1	0
Klebsiella pneum.ssp pneumoniae	96	84	1	65	97	0	70	99	94	98	90	90	100	94	99	100	100	100	0	95	0
Klebsiella pneum.ssp rhinoscleromatis	0	0	0	0	1	0	96	50	90	90	90	90	50	50	50	92	89	99	0	0	0
Kluyvera spp	80	70	90	0	50	0	83	85	90	50	0	50	85	80	60	100	95	98	81	4	0
Leclercia adecarboxylata	100	0	0	0	27	0	99	100	100	100	79	99	100	100	55	98	58	98	98	0	0
Moellerella wisconsensis	75	0	0	0	100	0	0	0	0	0	100	0	0	100	100	0	100	100	0	0	0
Morganella morganii	1	5	95	98	1	83	1	0	1	1	0	0	0	0	0	27	0	97	98	1	0
Pantoea spp 1	99	0	0	3	40	0	96	85	96	87	12	44	99	96	1	96	13	100	71	1	0
Pantoea spp 2	99	0	0	0	80	0	76	66	99	92	4	83	90	42	98	99	47	100	28	47	0
Pantoea spp 3	70	0	0	0	35	0	8	28	71	15	0	16	3	0	84	96	4	99	4	50	0
Proteus mirabilis	0	2	96	98	44	99	1	6	1	24	0	1	1	0	1	85	1	98	1	15	0
Proteus penneri	1	0	0	100	0	100	0	0	0	4	0	0	0	0	75	2	1	83	0	0	0
Proteus vulgaris	1	0	0	98	8	99	0	64	1	5	0	1	0	0	89	1	1	97	90	1	0
Providencia alcalifaciens	1	0	1	0	83	97	0	0	1	1	75	0	1	0	3	2	1	100	99	0	0
Providencia rettgeri	2	0	0	99	73	99	0	60	1	1	87	29	0	1	26	1	1	99	98	0	0
Providencia stuartii	2	0	0	34	67	96	0	5	1	1	1	0	1	0	13	96	1	98	83	0	0
Salmonella arizonae	92	99	92	0	85	0	93	0	82	99	0	98	0	23	0	84	0	100	0	0	0
Salmonella choleraesuis	0	99	92	0	7	0	0	0	8	39	0	69	0	5	0	28	0	100	0	0	0
Salmonella paratyphi A	0	0	91	0	11	0	0	0	99	11	0	98	0	7	0	99	0	100	0	0	0
Salmonella typhi	0	99	0	0	2	0	0	0	1	1	0	0	0	30	0	70	0	99	0	1	0
Salmonella spp	2	99	99	0	83	0	1	0	93	69	0	92	2	53	3	94	2	100	1	0	0
Serratia ficaria	82	0	0	0	100	0	0	100	55	0	0	0	0	0	95	96	0	100	0	3	0
Serratia fonticola	100	75	99	1	40	0	98	99	90	51	97	55	1	98	20	99	98	100	0	1	0
Serratia liquefaciens	88	76	94	1	66	0	1	84	47	26	3	1	10	23	97	99	74	100	0	70	0
Serratia marcescens	57	98	99	1	82	2	0	83	0	2	25	0	0	1	96	99	2	100	1	72	0
Serratia odorifera 1	90	97	81	1	90	0	0	98	66	66	1	5	75	91	100	99	99	100	66	11	0
Serratia odorifera 2	90	97	5	0	90	0	0	5	66	66	1	5	15	50	0	99	11	100	66	60	0
Serratia plymuthica	97	0	0	0	70	0	1	77	50	30	0	0	40	15	100	96	30	90	1	18	0
Serratia rubidaea	98	73	0	1	81	0	47	99	73	73	92	1	2	92	99	99	99	100	0	89	0
Shigella sonnei	82	0	99	0	0	0	0	0	99	6	0	65	0	1	0	99	2	100	0	0	0
Shigella spp	1	0	0	0	1	0	0	0	18	1	0	3	0	1	0	60	0	95	24	0	0
Yersinia enterocolitica	65	0	77	85	1	0	0	31	40	30	0	1	31	1	70	61	7	98	41	1	0
Yersinia pestis	61	0	0	1	0	0	0	99	1	1	0	1	0	1	0	1	0	100	0	0	0
Yersinia pseudotuberculosis	61	0	0	99	1	0	0	98	1	1	0	10	1	1	0	1	1	98	0	0	0
Vibrio alginolyticus	1	1	0	0	1	0	0	7	0	0	0	0	1	0	98	92	0	82	92	0	98
Vibrio cholerae	95	89	80	1	72	1	1	0	3	10	0	0	0	0	100	70	0	100	98	70	100
Vibrio fluvialis	40	0	0	0	16	0	0	3	93	0	0	0	30	1	100	100	0	100	13	0	100
Vibrio hollisae	9	0	0	4	0	0	0	0	65	0	0	0	4	0	0	4	0	96	100	0	100
Vibrio parahaemolyticus	0	2	2	4	14	40	0	0	84	0	0	0	0	0	0	99	0	100	99	0	100
Vibrio vulnificus	73	2	1	1	13	13	0	5	1	0	0	0	73	1	7	80	1	73	73	0	100
Photobacterium damsela	11	50	0	33	0	0	0	0	0	0	0	0	0	0	6	56	11	99	0	0	94
Plesiomonas shigelloides	100	100	100	0	0	0	0	0	0	0	0	0	0	1	0	99	0	99	100	0	100
Aeromonas hydrophila	92	19	0	0	41	1	0	26	26	1	0	8	41	1	96	99	0	98	99	59	100
Acinetobacter/Pseudomonas spp	0	2	0	0	65	2	2	0	39	41	0	0	0	19	2	0	0	36	0	0	19