



BBL[®] CRYSTAL[™]

IDENTIFICATION SYSTEM



Why to use BBL[®] CRYSTAL[™] IDENTIFICATION SYSTEM

- ▶ Covers all identification needs.
- ▶ Closed system with high safety.
- ▶ Easy to use system.
- ▶ Unique inoculation procedure.
- ▶ No reagents required.
- ▶ Reliable and accurate.



Introduction

- ▶ Micromethods for the biochemical identification of microorganisms were reported as early as **1918**.



Concept of BBL[®] CRYSTAL[™] ID SYSTEM

- ▶ Many of the tests used in the BBLCrystal are modifications of classical methods.
- ▶ These include tests for:
 - 1- Fermentation.
 - 2- Oxidation
 - 3- Degradation
 - 4- Hydrolysis of various substrates.
- ▶ In addition, there are **Chromogen** and **Fluorogen** linked substrates to detect enzymes that microbes use to metabolize various substrates.

BBL[®] CRYSTAL[™] ACCESSORIES

Device/Acc.	Function
BBL [®] Crystal [™] Panel Viewer	Panel Viewer with combined white & UV light for interpretation of chromogenic & fluorogenic reactions
BBL [®] CrystalSpec [™] Nephelometer	A battery powered instrument for correct adjustment of McF standards (0,5 und 4,0).
BBL [®] Crystal [™] MIND Database	Electronic, interactive Codebook for data interpretation and reviewing the results.
BBL [®] Crystal [™] Autoreader	Automatic reading of the Crystal panels in connection with the Crystal MIND Database .

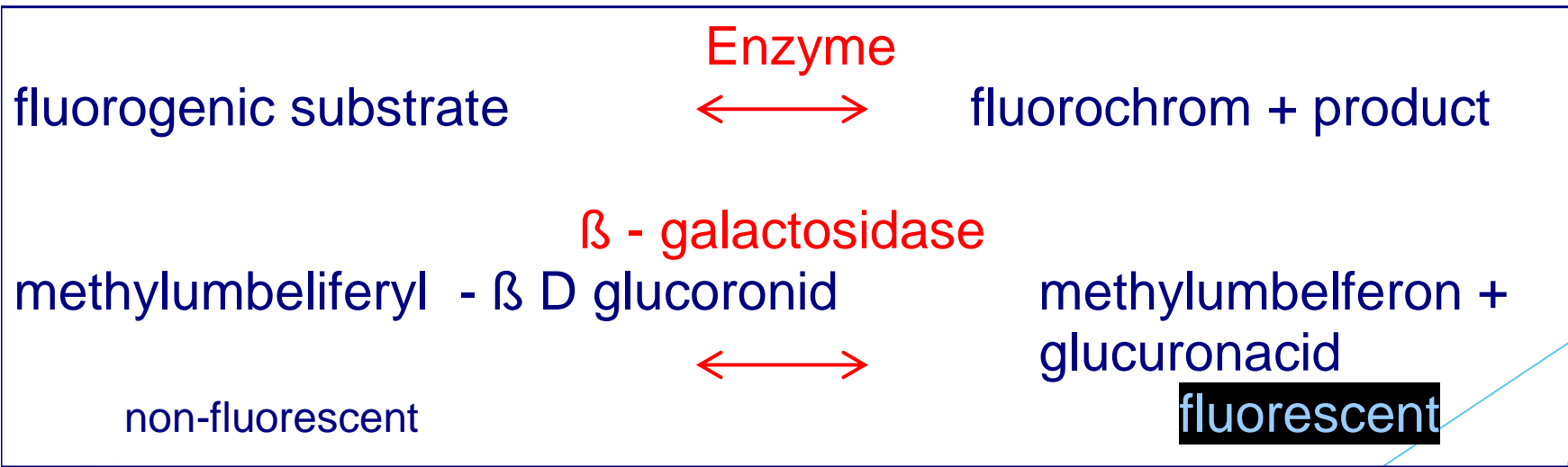
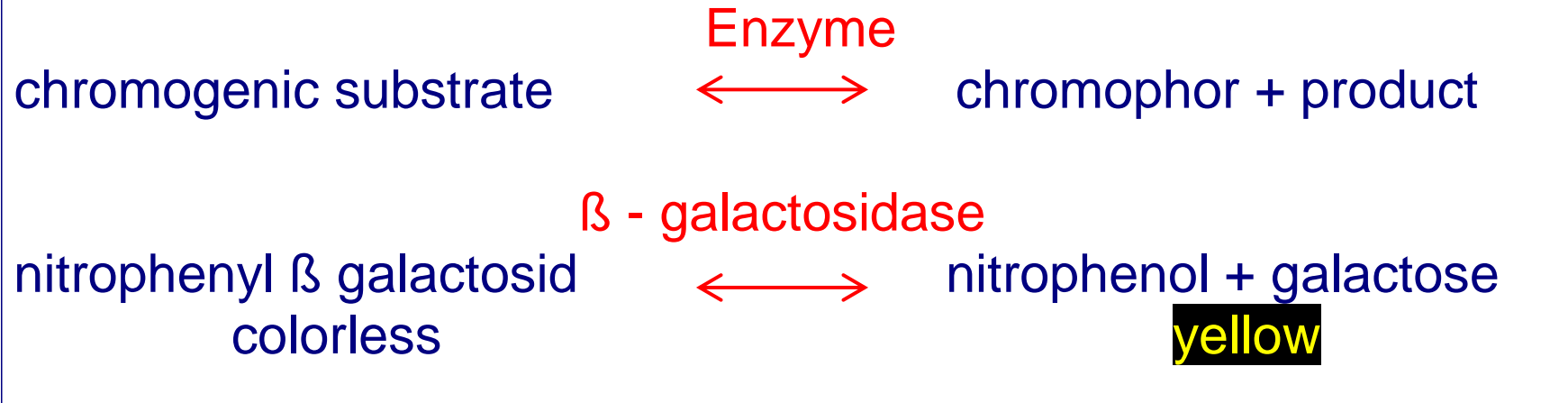


BBL[®] CRYSTAL[™] TECHNOLOGY

- An identification system with fluorogenic and chromogenic substrates for detection of enzymes, which are used by microorganisms for metabolism.
- Some substrates are modifications of classical methods (sugar, aminoacids,etc) other substrates are more unusal.

BBL[®] CRYSTAL[™] TECHNOLOGY

CHROMOGENIC & FLUOROGENIC SUBSTRATES



BBL® CRYSTAL™ TESTKITS

- BBL® Crystal™ **E/NF**
Test system for identification of Enterobacteriaceae and non-fermentive Gram -ve bacilli .
- BBL® Crystal™ GP
Test system for identification of Gram +ve cocci and Gram +ve bacilli.
- BBL® Crystal™ **RG/P**
Rapid-testsystem for identification of Gram +ve cocci and Gram +ve bacilli.
- BBL® Crystal™ **ANAEROB**
Test system for identification of Anaerobic organisms
- BBL® Crystal™ **N/H**
Test system for identification of Neisseria / Haemophilus

BBL® CRYSTAL™ IDENTIFICATION SYSTEMS

BBL® Crystal™ Database - Taxonomie

- BBL Crystal **E/NF** 123 SPECIES - 40 GENERA
- BBL Crystal GP 121 SPECIES - 24 GENERA
- BBL Crystal **R/GP** 92 SPECIES - 19 GENERA
- BBL Crystal **Anaerob** 108 SPECIES - 25 GENERA
- BBL Crystal **N/H** 37 SPECIES - 11 GENERA

BBL® CRYSTAL™ KITS

Presentation & Packaging:

- 20 lids with 29 or 30 dehydrated substrates.
- 20 bases with 30 wells(pores).
- 20 tubes with inoculum fluid (*each tube contains $2,2 \pm 0,1$ ml*)
- 2 incubation trays
- 1 chart for entering the substrate reactions

Color charts for comparing substrate reactions, delivered together with the Panel-Viewer



Materials Not Provided with the kits & must/ may be needed

- ▶ Sterile cotton swabs (do not use polyester swabs)
- ▶ Incubator (35 - 37° C) non-CO2 (40 - 60% humidity)
- ▶ McFarland standards.
- ▶ Nephelometer.
- ▶ Vortex
- ▶ BBLCrystal™ Panel Viewer or Autoreader
- ▶ BBLCrystal ID System Electronic Codebook or BBLCrystal ANR Manual Codebook
- ▶ BBL DMACA Indole Reagent Droppers & BBL Oxidase Reagent Droppers
- ▶ Culture plates
- ▶ Catalase reagent.

BBL CRYSTAL™

Identification Procedure in 4 easy steps

- ▶ 1. Inoculum preparation McFarland: 0.5 - 2.0 - 3.0 - 4.0
- ▶ 2. Inoculation of the panels
- ▶ 3. Incubation of the panels 4 or 18-24 hours
- ▶ 4. Reading & Interpretation

Before You begin

- ▶ BBLCrystal ID Systems are not for use directly with clinical specimens.
- ▶ Use isolates from media as specified in the Kit's insert.
- ▶ Use of selective media is also acceptable.
- ▶ Media containing esculin should not be used.
- ▶ The isolate must be a pure culture, no more than 18:24 h old.
- ▶ Some polyester swabs may cause problems with inoculation.
- ▶ Once lids are removed from the sealed pouches, they must be used within 1 hour.
- ▶ If the kit or any of the components are stored refrigerated, each should be brought to room temperature prior to use.

BBL CRYSTAL™ INOCULUM PREPARATION

- Colonies from the same morphology
- Use Cotton-tipped swab or plastic loop
- Aerobic bact.- no more, than 24 hours old isolate
- Anaerobic bact. - 24-48 hours generally
 - up to 72 hours for some slow growing cocci
 - up to 72-96 hours for Actinomyces
- CrystalSpec - Nephelometer
 - 0.5 McFarland : E/NF; Gram Pos.
 - 2.0 McFarland : Rapid Gram Pos.
 - 4.0 McFarland : Anaerobe
- Vortexing 10-15 sec., homogenous inoculum susp.



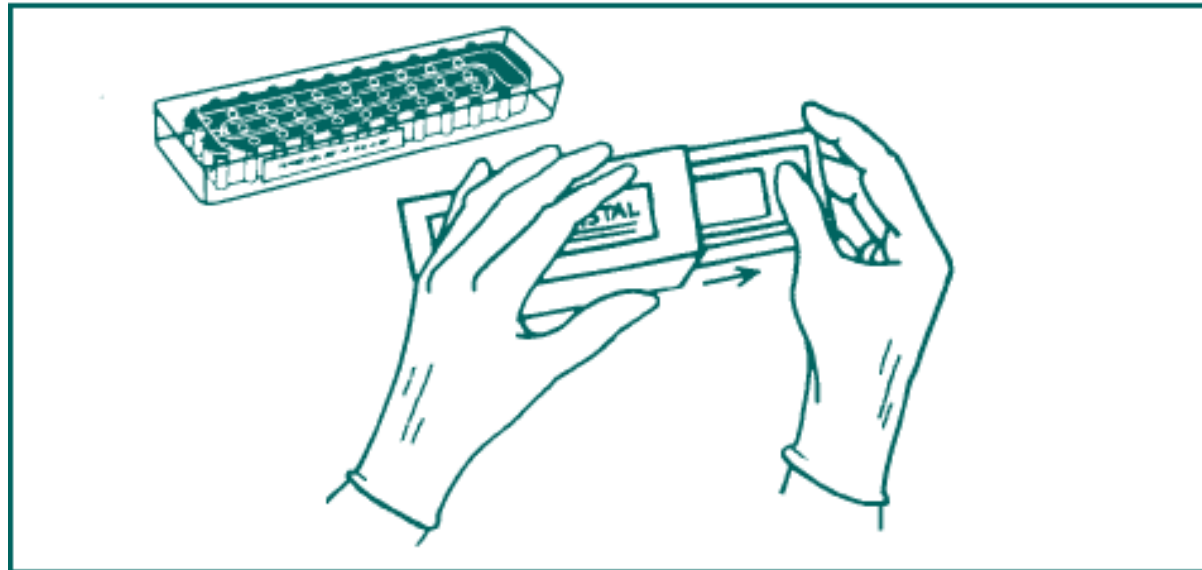
BBL® CRYSTAL™ MEDIA USAGE

Medium	E/NF	GP	RGP	N/H *	ANA
TSA 5% SB	X	X	X	X	
Columbia	X	X	X	X	X
Mc Conkey	X				
CNA		X	X		
Chocolate				X	
Schaedler					X
CDC					X
Brucella					X
Blood	X		X	X	X

* Selective media:GC - Lect; Martin Lewis; Mod. Thayer Martin;
New York City or V - Agar can also be used

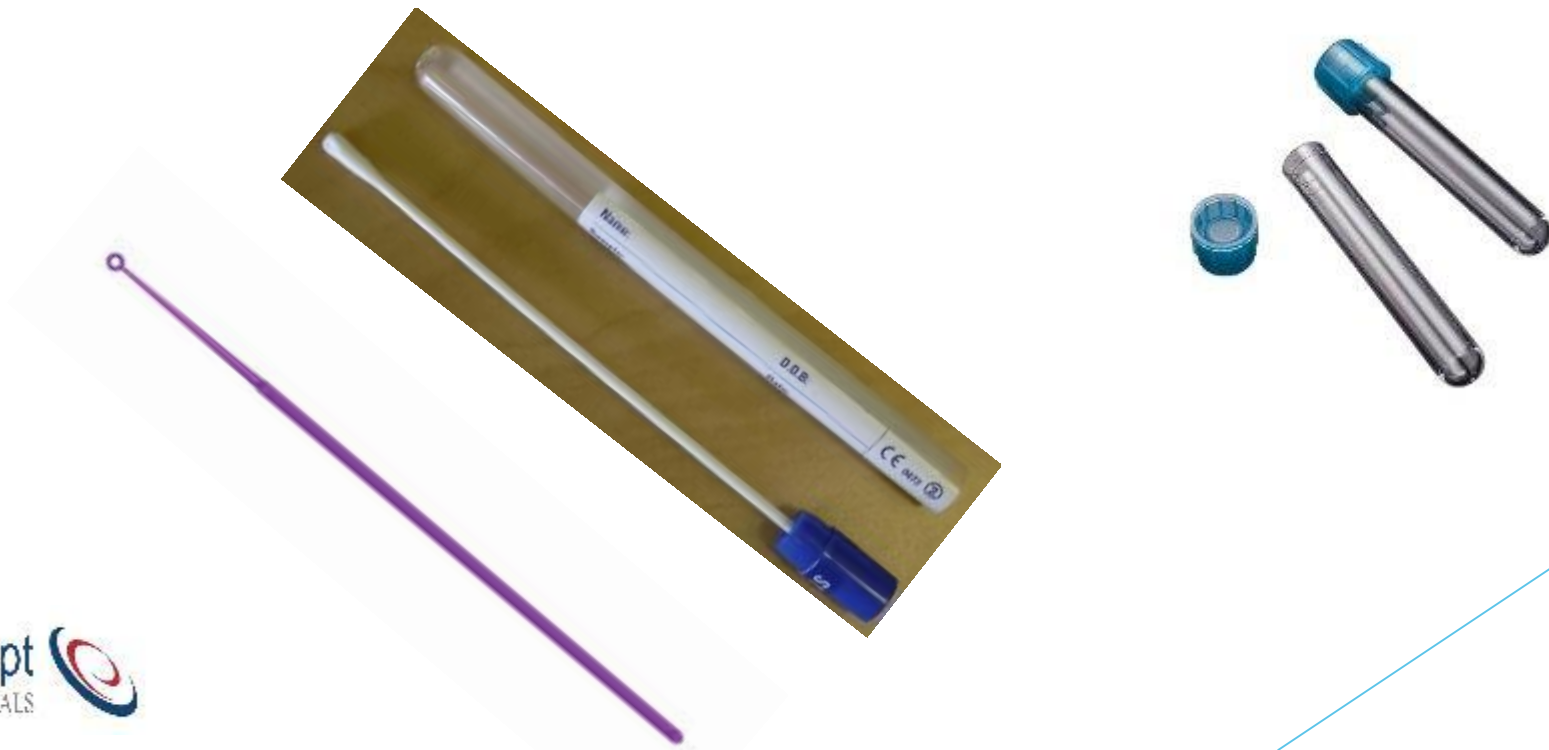
1

- ▶ Lid is removed from the pouch and desiccant is discarded.
- ▶ It must be used within 1 hr of removal from pouch (Shouldn't be used if there is no desiccant)



2:

- ▶ Inoculum fluid tube is to be labeled with the specimen number.
- ▶ Specimen is to be taken with a cotton swab or disposable plastic loop by an aseptic technique.



3

- ▶ Colonies are then suspended in the Inoculum Fluid.
- ▶ Tube is recapped and vortexed for 10-15 secs; McFarland standard that is set depends on the kit.



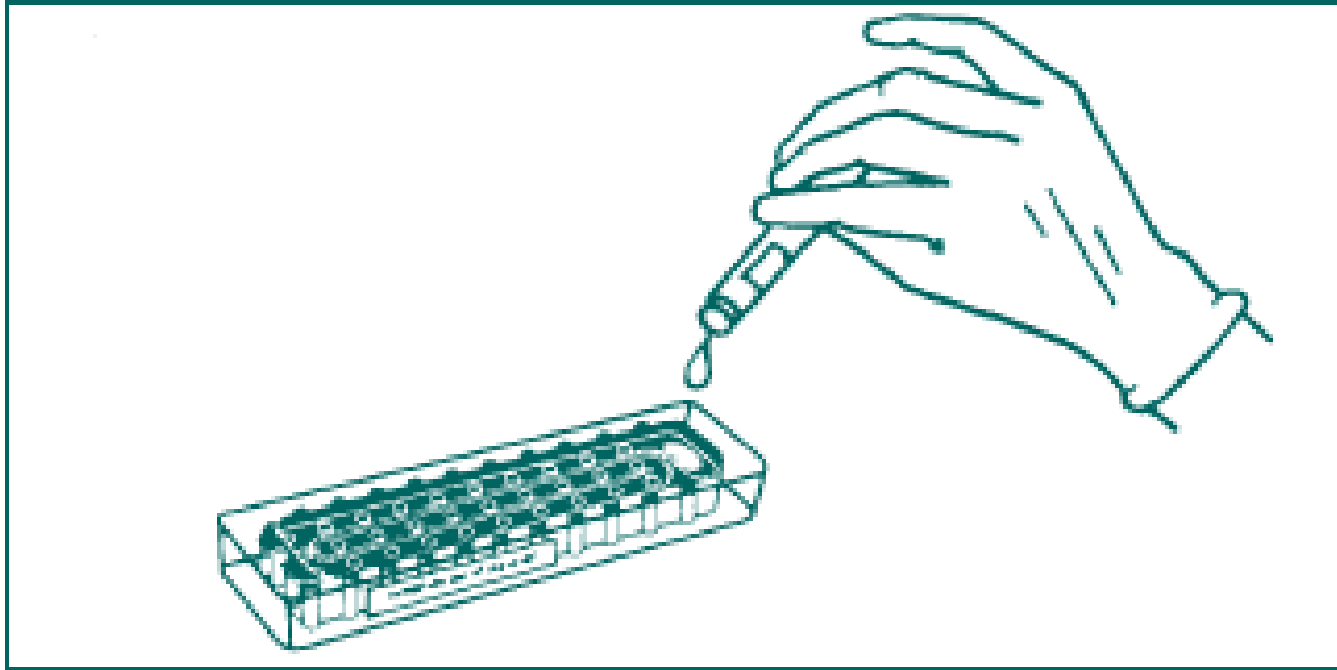
Adjust McF value to the standard

- ▶ If the inoculum suspension concentration is lower or in excess of the recommended McFarland standard:
 - 1- Use a fresh tube of inoculum fluid to dilute the inoculum suspension or use 0.85% sterile saline to dilute the inoculum.
 - 2- Concentrate the inoculum by adding more from the specimen using the swab.
- ▶ Remove the excess amount added to the tube with a sterile pipet so that the final volume of inoculum fluid is approximately equivalent to that of the original volume in the tube (2.3 ml)



4

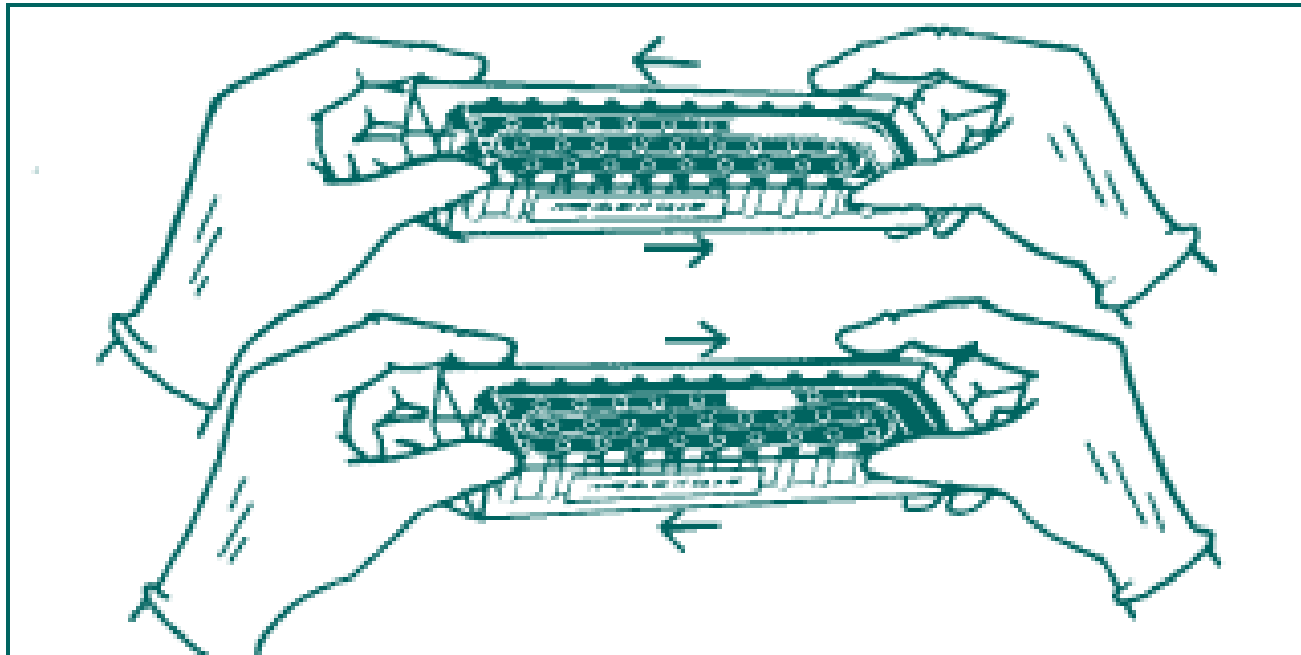
- ▶ Take the base, and mark the specimen number on the side wall
- ▶ Pour entire contents of inoculum fluid into target area of the base.



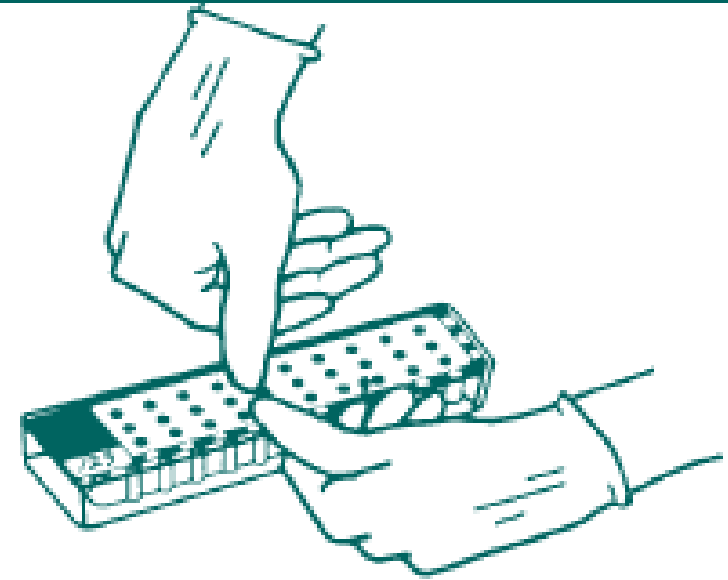
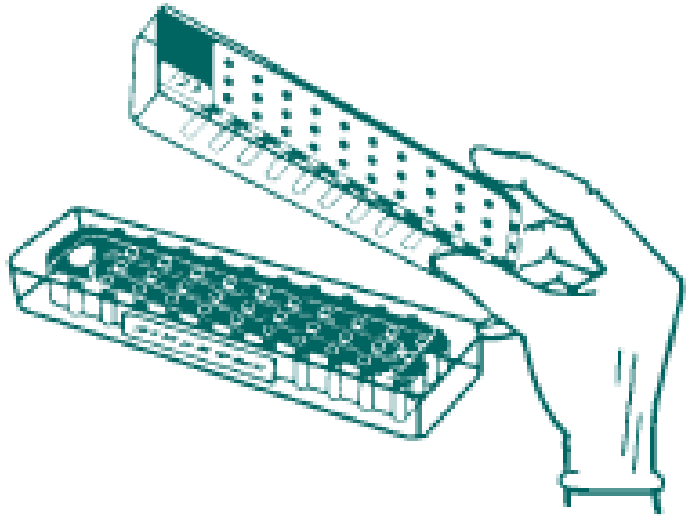
5

- ▶ Hold the base in both hands and roll inoculum gently along the tracks until all of the wells are filled.
- ▶ Roll *back* any excess fluid to the target area and place the base on a bench top.

The inoculum should be slowly rolled across the tracks to ensure a proper fill of all wells.

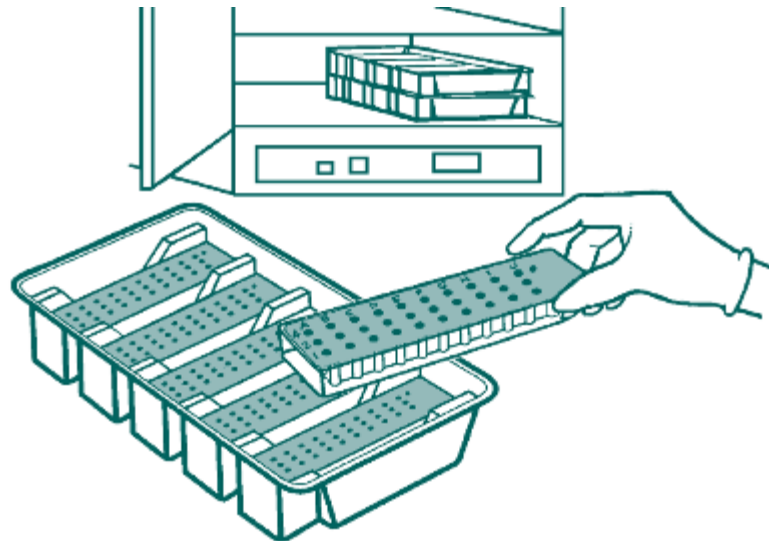


- 6
- ▶ Align the lid so that the labeled end of the lid is on top of the target area of the base.
 - ▶ Push down until a slight resistance is felt. This leads to rehydration of substrate to initiate reaction.
 - ▶ Let 5 min. in face-up position.



7

- ▶ **Incubation:** Place inoculated panels in incubation trays. Ten panels can fit in one tray (5 rows of 2 panels).
- ▶ All panels should be incubated **face down** (larger windows facing up)

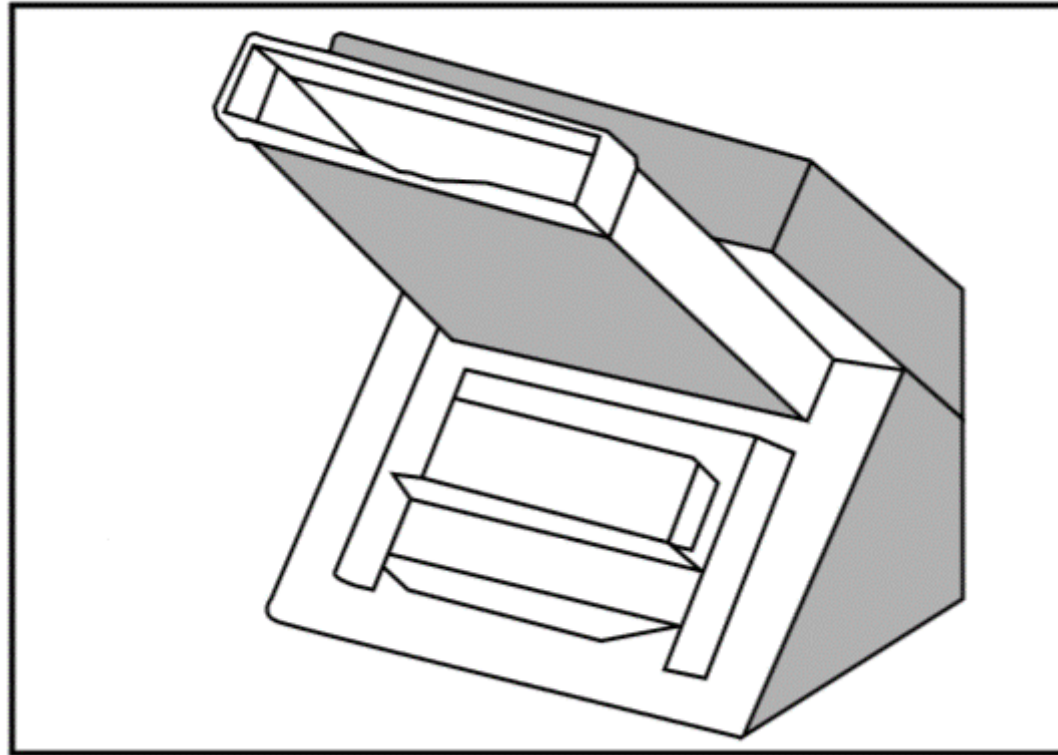


BBL[®] CRYSTAL[™] TESTCONDITIONS

Crystal Kit	Mc. Farland Std	Inkubationtime	Temperature °C
E/NF	0.5	18 -24 h	35 - 37
GP	0.5	18 -24 h	35 - 37
R/GP	2.0	4h	35 - 37
N/H	3.0	4h	35 - 37
ANA	4.0	4h	35 - 37



▶ Reading the results



Before Reading

BBL® CRYSTAL™ additional needed information

For correct identification with the electronic database to be entered together with the profile number

Test	E/NF	GP	RGP	N/H	ANA
gram stain		X	X	X	X
morphologie		X	X	X	X
indol	(X)				X
oxidase	X				
catalase					X

BBL[®] CRYSTAL[™] READING

- After the incubation time place the panels on the Panel Viewer.
- Check substrate reactions on white (and UV-) light or use the Auto Reader.



BBL[®] Crystal[™] Panel Viewer



BBL[®] Crystal[™] Auto Reader

BBL[®] CRYSTAL[™] READING using the panel viewer

- Compare the panel reactions with the color chart and notice the actual panel reactions
- Each reaction is linked to a value (1, 2 or 4)
- Adding the values by column results in a 10 digit profile number
- The profile number is manually entered in the database

Example:	A	B	C	D	E	F	G	H	I	J
4	*	+	-	-	+	+	+	-	+	-
2	-	+	+	+	-	+	-	+	+	-
1	+	-	+	-	+	-	-	+	+	-
Profile	1	6	3	2	5	6	4	3	7	0

*(4A) = fluorescent negative control

- ▶ Panels are read with the larger window facing down using the color chart and BBL Crystal Report pad.
- ▶ Columns G-J are read with a regular (white) light source
- ▶ Columns A - F are read with a fluorescent light source



BBL CRYSTAL™ Reading using the AUTOREADER

Benefits of using the Autoreader

- ▶ Combined white & UV light
- ▶ Eliminate the subjectivity of the manual reading
- ▶ Reference panel to check the light sources
- ▶ Automatic switch-off after 1 hour
- ▶ Only one moving part



BBL® CRYSTAL™ QUALITY CONTROL STRAINS

Crystal kits	control strains	ATCC number
E/NF	<i>Klebsiella pneumoniae</i>	ATCC 33495
GP	<i>Streptococcus pyogenes</i>	ATCC 19615 X
R/GP	<i>Streptococcus pyogenes</i>	ATCC 19615 X
N/H	<i>Moraxella catarrhalis</i>	ATCC 25240
ANA	<i>Bacteroides fragilis</i>	ATCC 25285 X

X Microtrol strain from BD

Warning

- ▶ After use, all infectious materials including plates, cotton swabs, inoculum fluid tubes, and panels must be autoclaved prior to disposal or incineration.





BBL CRYSTAL MIND

Software

GENERAL WORKFLOW

Reading and Data-entering

Identification

Viewing and validation of results

Reporting of results



BBL CRYSTAL MIND

The screenshot shows the BBLCrystal Microbiology Interactive Database [Data Entry] window. The interface includes a menu bar (File, Batch ID, Review, Reports, Setup, Percentage) and a main data entry area. On the left, there is a vertical toolbar with buttons for Add, Clear, ID, and Close. The main area contains fields for Accession # (622), Patient Id (640907), and Patient Name (Takacs Judit). Below these are buttons for various tests: ENF, RSE, ANR CDC, ANR SCH, ANR ALT, GP, RGP, and NH. A central panel displays a grid of test results for 4, 2, and 1 samples, with columns labeled A through J. A yellow question mark is visible in the 'E' column of the first row. To the right of the grid is a 'Profil' section with a keyboard entry indicator and a 'Virtual field' for mouse entry. At the bottom, there are 'Off-line tests' including Gram (+ Bacilli, + Cocci, ?). Annotations with arrows point to various parts of the interface: 'Delete' points to the toolbar, 'Data Entry' points to the main form, 'Type of Panels' points to the test buttons, 'Profil' points to the keyboard entry section, 'Virtual field' points to the mouse entry section, and 'Off-line tests' points to the Gram test options.

Delete

Data Entry

Type of Panels

Profil

Virtual field

Off-line tests

BBL CRYSTAL MIND

BBLCrystal Microbiology Interactive Database [Data Entry]

File Batch ID Review Reports Setup Percentage

Accession #
622

Patient Id 640907 **Patient Name** Takacs Judit

ENF RSE ANR ANR ANR GP RGP NH
CDC SCH ALT

	A	B	C	D	E	F	G	H	I	J
4	○	⊕	⊕							
	FCT	FPH	FTR	FHD	TRE	SUC	ARA	BGL	PHO	URE
2	⊖	⊖	⊖							
	FGC	FGS	FAR	FGN	LAC	MNT	GLR	PCE	PAM	ESC
1	⊖	⊕	⊖							
	FVA	FPY	FGA	FIS	MAB	MTT	FRU	PLN	PGO	ARG

Gram
 + Bacilli
 + Cocci
 ?

Keyboard Entry
 Mouse Entry

Complete Panel

Virtul field
and profil
are moving
together

BBL CRYSTAL MIND

Specimen report

Suppl. tests

Back to data-entry

Panel	Profile	Patient Name	Accession Number
ENF	4211144444	Harry Potter	12345

Current Record	Biotype Validity	Confidence
[1] Klebsiella pneumoniae ssp pneumoniae	1039	.4758
[2] Serratia rubidaea	211	.2425
[3] Enterobacter cloacae	660	.2055

Statistics
The Crystal ID Report is based on these statistics. Choosing an organism of the basis of these statistics is not recommended.

Message
The Crystal ID system reports these choices. Supplemental testing is recommended.

BBL Crystal Enteric/NF 4.0

Profile: 6765677557 Oxidase: - Indole: -

Worklist

Results-field

Special messages

BBL CRYSTAL MIND

Supplemental Test							
Organism Name	VP	MO	CB	GE	DN	MR	OR
Enterobacter cloacae	99	95	99	1	1	5	96
Klebsiella pneumoniae ssp pneumoniae	98	1	98	1	1	10	1
Serratia rubidaea	99	85	94	90	99	20	1

Description of the Test Codes and Results	
42 : Growth at 42 degrees Celsius	
CB : Cellobiose	
DN : DNase	
GE : Gelatin	
HS : H2S	
MO : Motility	

<<>> Close All Organisms

Percentages of positivity

Explanation for abbreviations

BBL CRYSTAL MIND

Modification
of results

BBLCrystal Microbiology Interactive Database [Data Entry]

File Batch ID Review Reports Setup Percentage

Accession #
82372

Patient Id
10023

Patient Name
JOHN EDWARD PAGE

Modify

ENF RSE ANR ANR ANR GP RGP NH
CDC SCH ALT

	A	B	C	D	E	F	G	H	I	J
4	+	+	+	+	+	+	+	+	+	+
	ARA	MNS	SUC	MEL	RHA	SOR	MNT	ADD	GAL	IND
2	+	+	+	-	+	+	+	+	-	-
	PHO	BGL	NPG	PRO	BPH	BXY	AAR	PHC	GLR	NAG
1	-	+	-	+	-	+	+	+	+	+
	GGL	ESC	PHE	URE	GLY	CIT	MLO	TTC	ARG	LYS

Close

Oxidase
 +
 -

Indole
 +
 -
 ?

Keyboard Entry
Mouse Entry

A B C D E F G H I J
6 7 6 5 6 7 7 5 5 7

BBL CRYSTAL MIND

	Biotype Validity	Confidence
[1] <i>Vibrio hollisae</i>	3199	.532
[2] <i>Weeksella virosa/Bergeyella zoohelcum</i>	63113	.3479
[3] <i>Shigella dysenteriae</i>	1381	.0873

Organism information

BBLCrystal Microbiology Interactive Database [Review]

File Data Entry Batch ID Reports Setup Percentage Reference Test

Klebsiella pneumoniae ssp pneumoniae

Notes

¶ 3 5 : Important cause of nosocomial and community-acquired infections. Associated with lobar pneumoniae, urinary and biliary tract infections, wounds and bacteremias. Virtually limited to immunocompromised persons.

2 : Microbiological Terminology Update - Enterobacteriaceae, Copyright, 1986 Hoffman-LaRoche Laboratories

3 : Balows, A., W.J. Hausler, Jr., K.L. Herrman, H.D. Isenberg, Manual of Clinical Microbiology, 5th Edition. American Society for Microbiology, Washington, D.C.

5 : Mandell, G.L. R.G. Douglas, Jr. and J.E. Bennett. 1990. Principles and Practice of Infectious Diseases, 3rd. Edition. Churchill Livingstone Inc., New York.

Nomenclature

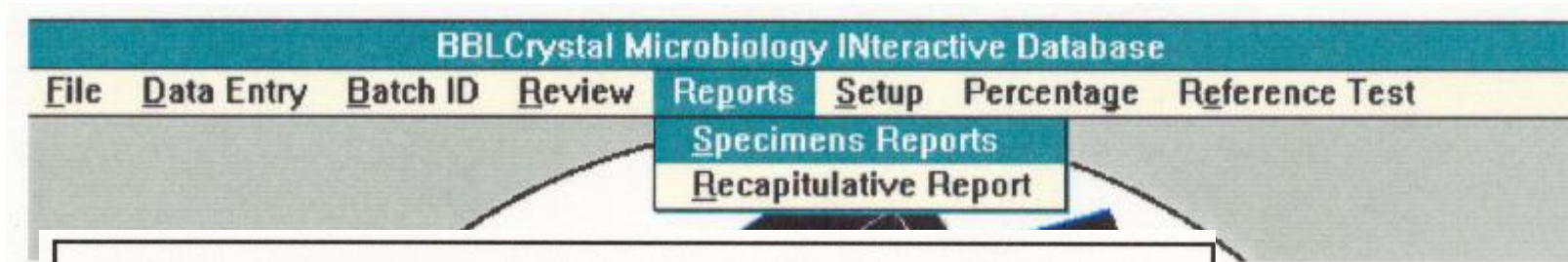
Previously known as: *Klebsiella pneumoniae*

Atypical reactions for this organism in the system :

+ : / PNP N-Acetyl-Beta-D-Glucosaminide
- : / Gamma-L-Glutamyl-P-NA

Review

BBL CRYSTAL MIND



BBL Crystal MIND - Specimen Report
4001599 11:48:06

Accession #: 82372
Patient Id: 10023
Patient Name: JOHN MEDWARDPAGE
Profile: 6765677557 *BBL Crystal EstriNF 4.0*

Indole: -
Oxidase: -

Biotype: Confidence Factor:
Klebsiella pneumoniae 1039 0.4758
Serratia chelonae 211 0.2425
Enterobacter cloacae 660 0.2055

Statistics: The Crystal ID Report is based on these statistics. Choosing an organism of the basis of these statistics is not recommended.
Message Report: The Crystal ID system reports these choices. Supplemental testing is recommended.

	NI	WP	MD	RV	OR	GE	EM	PR	PH	MS	OR	MG
KLEPNEP	99	98	1	99	98	1	1	99	?	1	1	?
SERRFUS	99	99	99	99	99	99	99	99	?	1	1	?
ENTOLO	99	99	95	99	99	1	1	5	?	1	96	?
RARDL												

Final Id:

Typical Reaction:

	ARA	PHO	GGL	MNS	BGL	ESC	SUC	NFG	PHE	MEL	PRO	URE	RHA	BPH	GLY	SOR	BIV	CI	MNT	WAR	MLO	ADO	PHC	TTC	GAL	GLR	ARG	IND	NAG	LVS
KLEPNEP				-																										*
SERRFUS				-									*							*										
ENTOLO				-																								*		

BBL CRYSTAL MIND

Recapitulative Report

Result Date Range

From To

Giving of data range

BBL Crystal MIND - Recapitulative Report 4/30/1999 > 4/30/1999
4/30/1999 12:03:37

Accession #:	Patient Name:	Profile:	Gram:	Indole:	Oxidase:	Catalase:	Organism:
57688	PAUL CAMPOGNONE	2664002673 GP	+ Bacilli				BAC SPE CORAGU
82372	JOHN EDWARD PAGE	6765677557 ENF		-	-		KLEPNEP SERRIUB ENTCLO
+ 83445	ERIC DEFOUR	0000002000 ENF		+	+		
QC TEST		7777777777 ENF		+	+		QC1T1E QC2T1E

List of results within the given range of time

THANK YOU



Dr. Emad Reda