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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.

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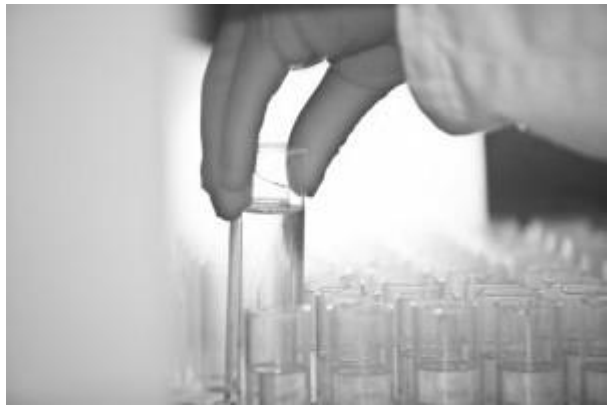
Introduction

- 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 .

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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.

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BBL® CRYSTAL™ TECHNOLOGY

CHROMOGENIC & FLUOROGENIC SUBSTRATES

Enzyme

chromogenic substrate ⟷ chromophor + product

ß - galactosidase

nitrophenyl ß galactosid ⟷ nitrophenol + galactose

colorless yellow

Enzyme

fluorogenic substrate ⟷ fluorochrom + product

ß - galactosidase

methylumbeliferyl - ß D glucoronid ⟷ methylumbelferon + glucuronacid

non-fluorescent fluorescent

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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

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BBL® CRYSTAL™ IDENTIFICATIONSYSTEMS

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

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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 ± 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

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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.
- Nephlometer.
- 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

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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 inuculum susp.

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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

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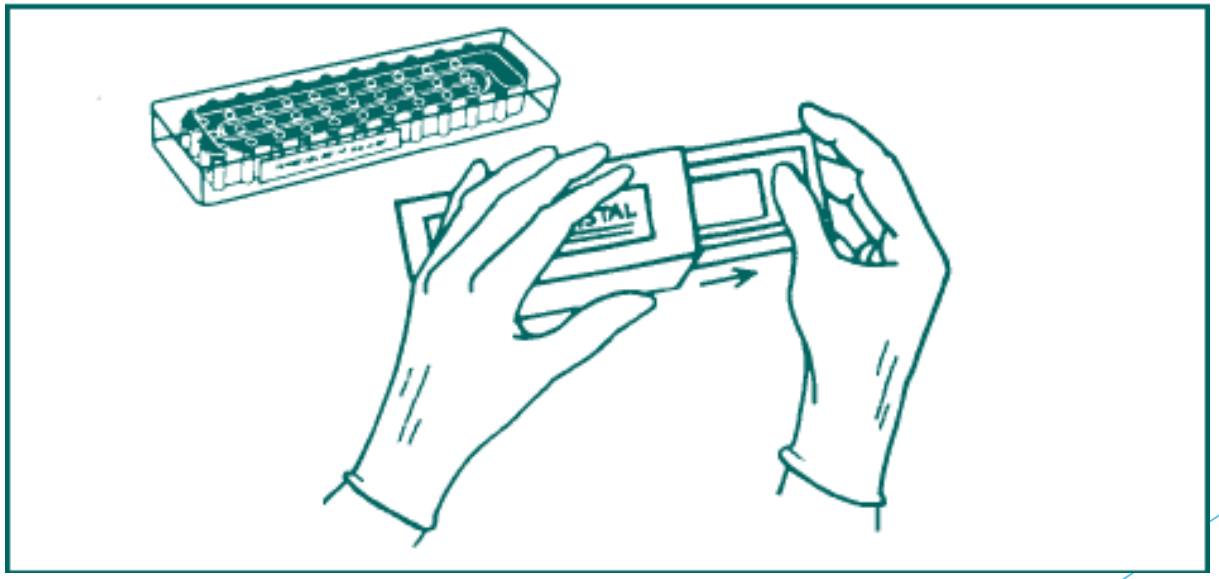
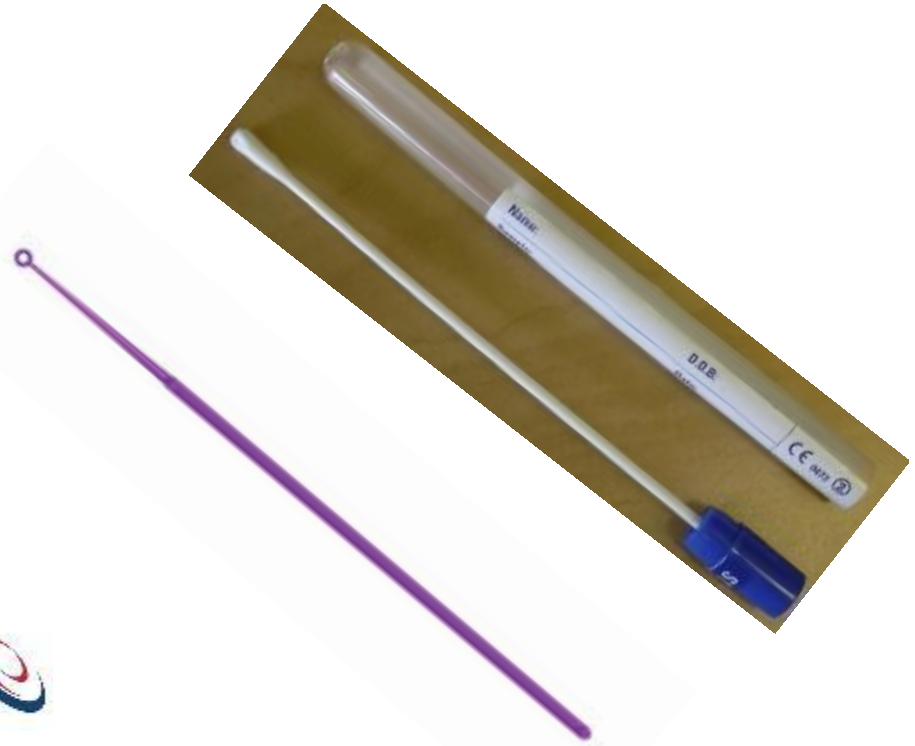
1:

- 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:

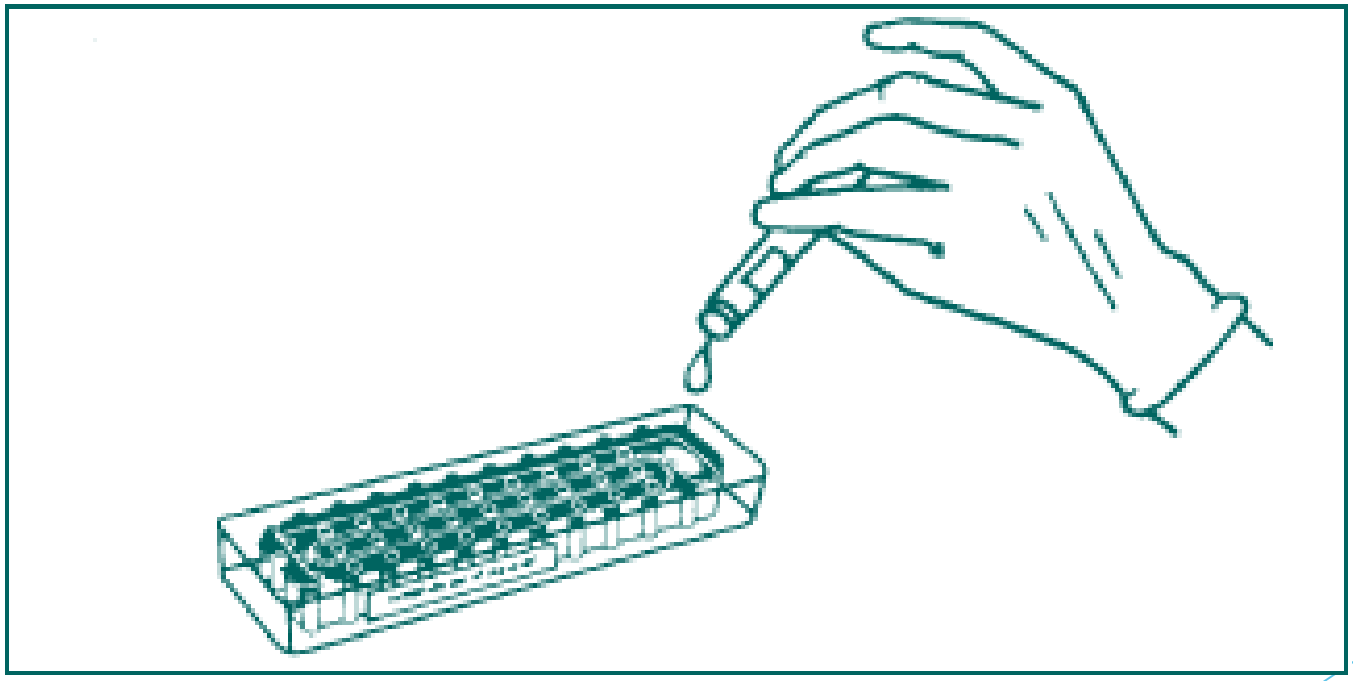
1- Use a fresh tube of inoculum fluid to dilute the inoculum suspension or use 0.85% sterile saline to dilute the inoculum.

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:

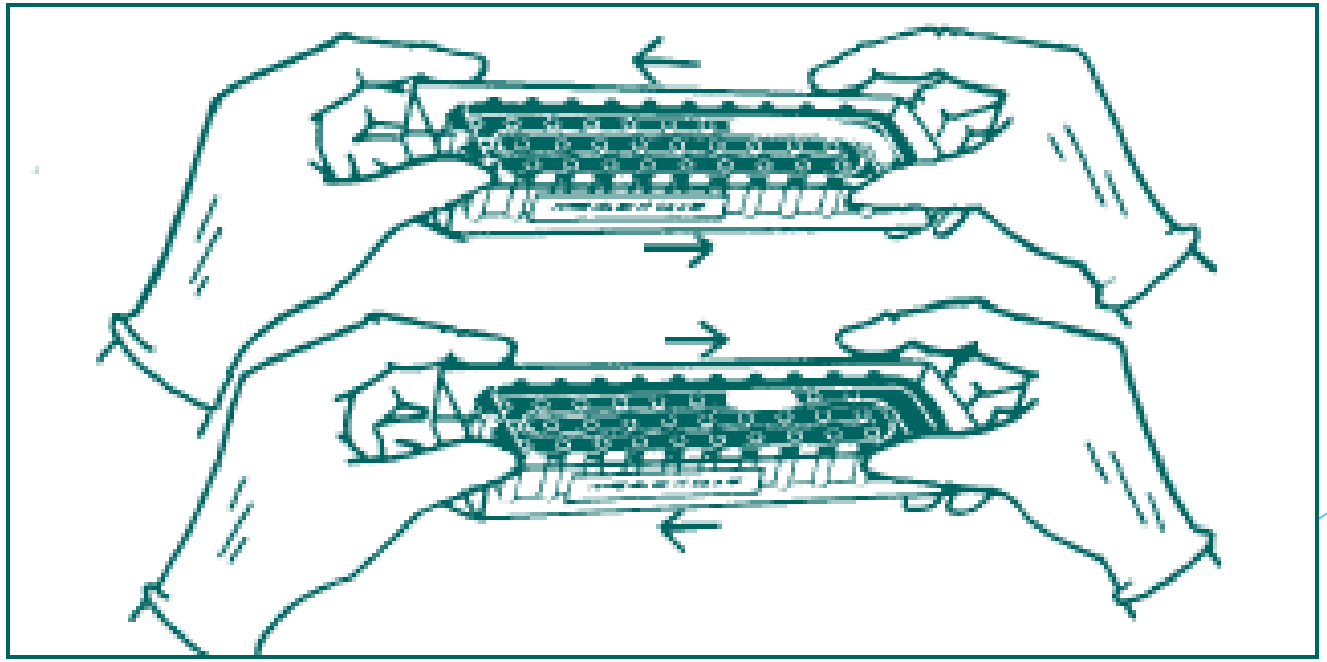
- Take the base, and mark the specimen number on the side wall
- Pour entire contents of inoculum fluid into target area of the base.



5:

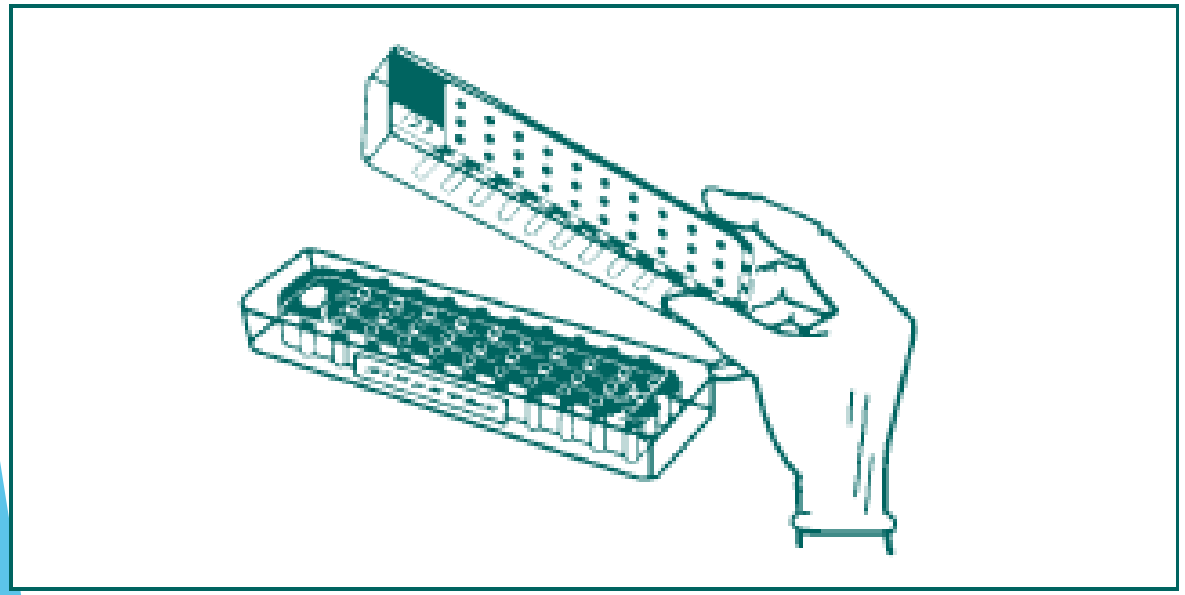
- Hold the base in both hands and roll inoculum gently along the tracks until all of the wells are filled.
- Roll *back* any excess fluid to the target area and place the base on a bench top.

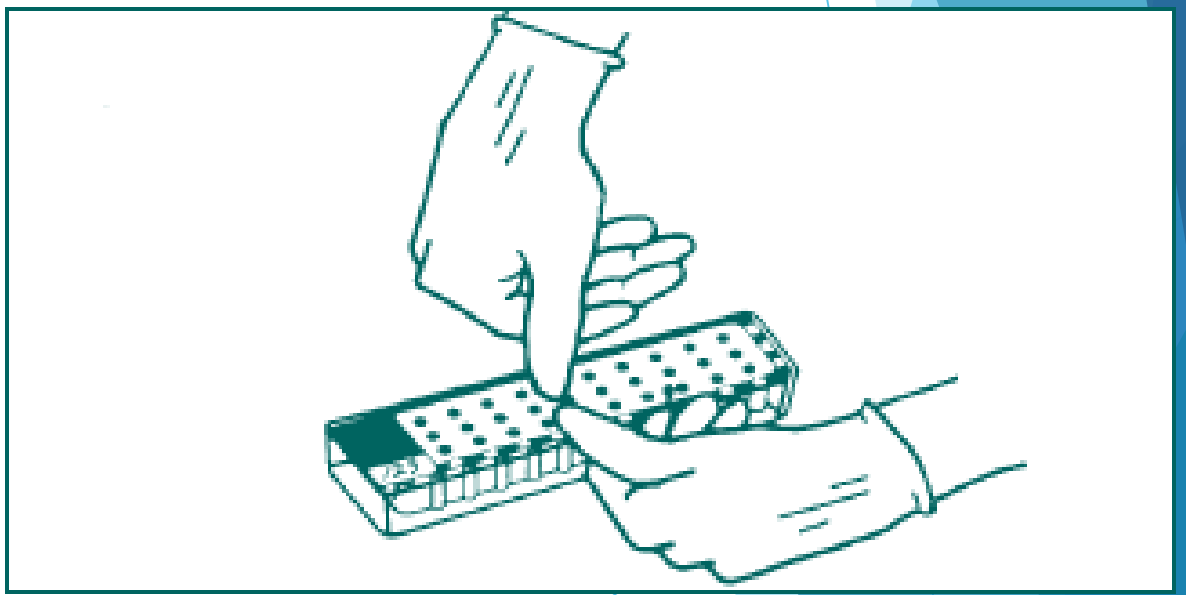
The inoculum should be slowly rolled across the tracks to ensure a proper fill of all wells.



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.





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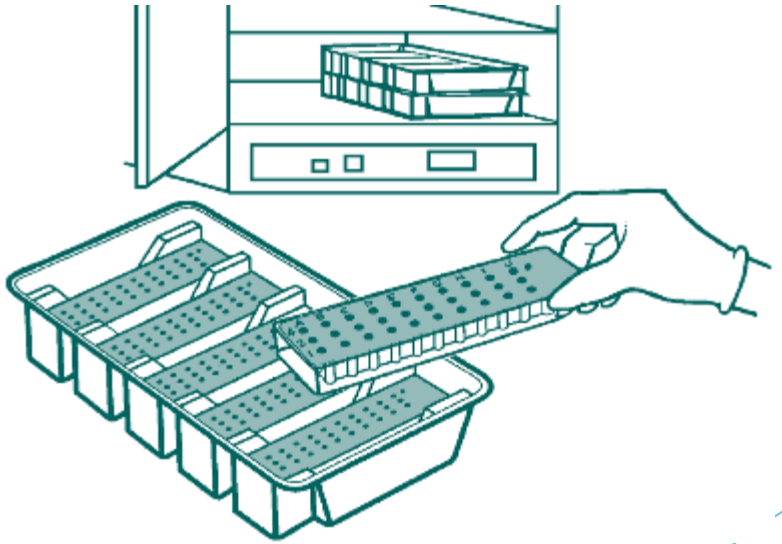
7:

- ***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

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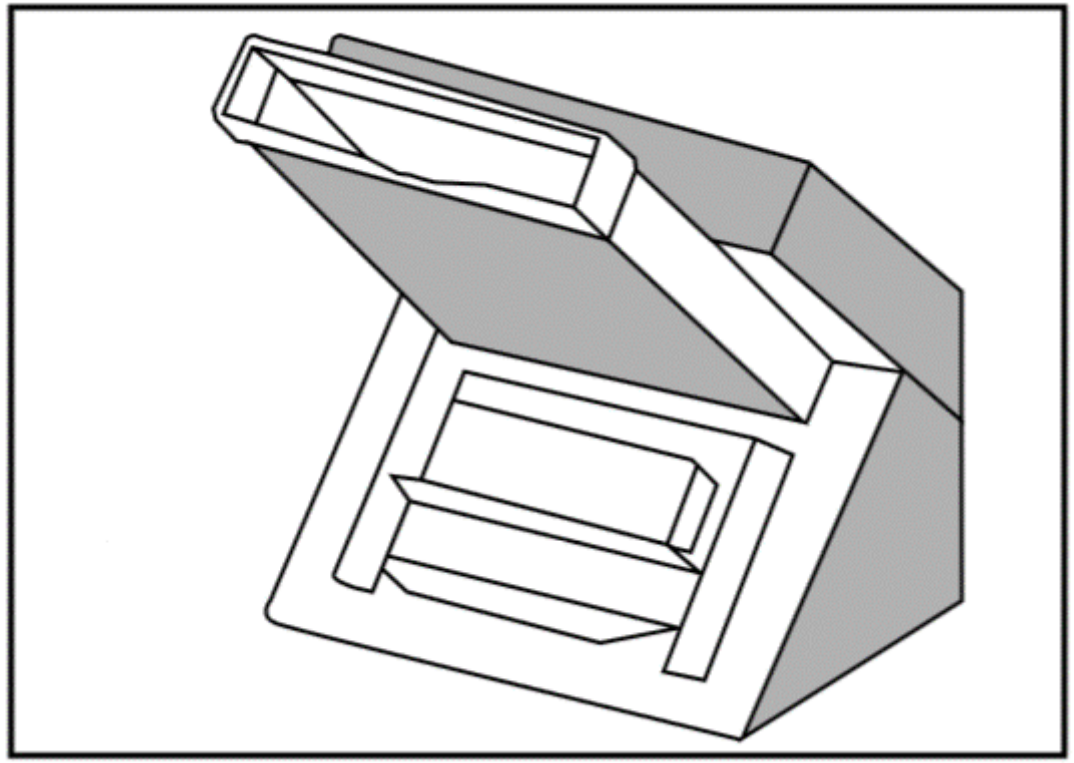
8: Reading the results



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

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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

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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 manualy 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

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- 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

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BBL® CRYSTAL™ QUALITY CONTROL STRAINS

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

X Microtrol strain from BD

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Warning

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

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BBL CRYSTAL MIND

Software

GENERAL WORKFLOW

Reading and Data-entering

Identification

Viewing and validation of results

Reporting of results

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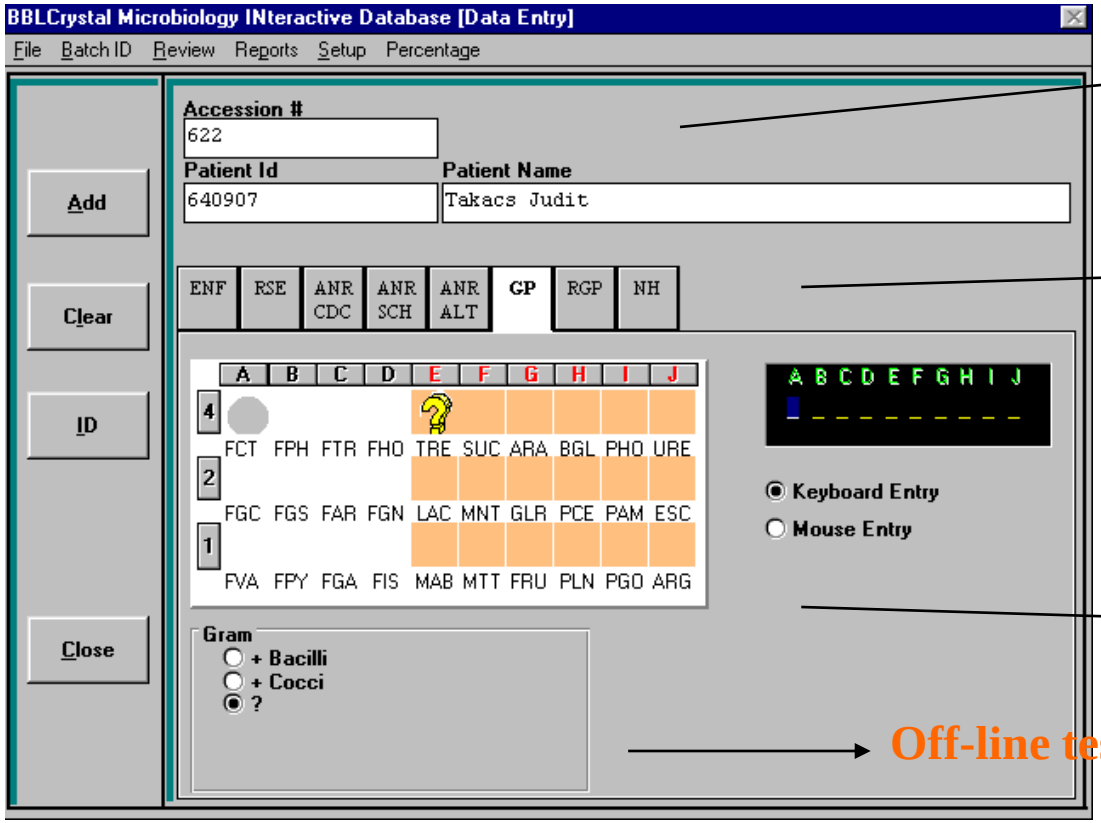


Data Entry

Type of Panels

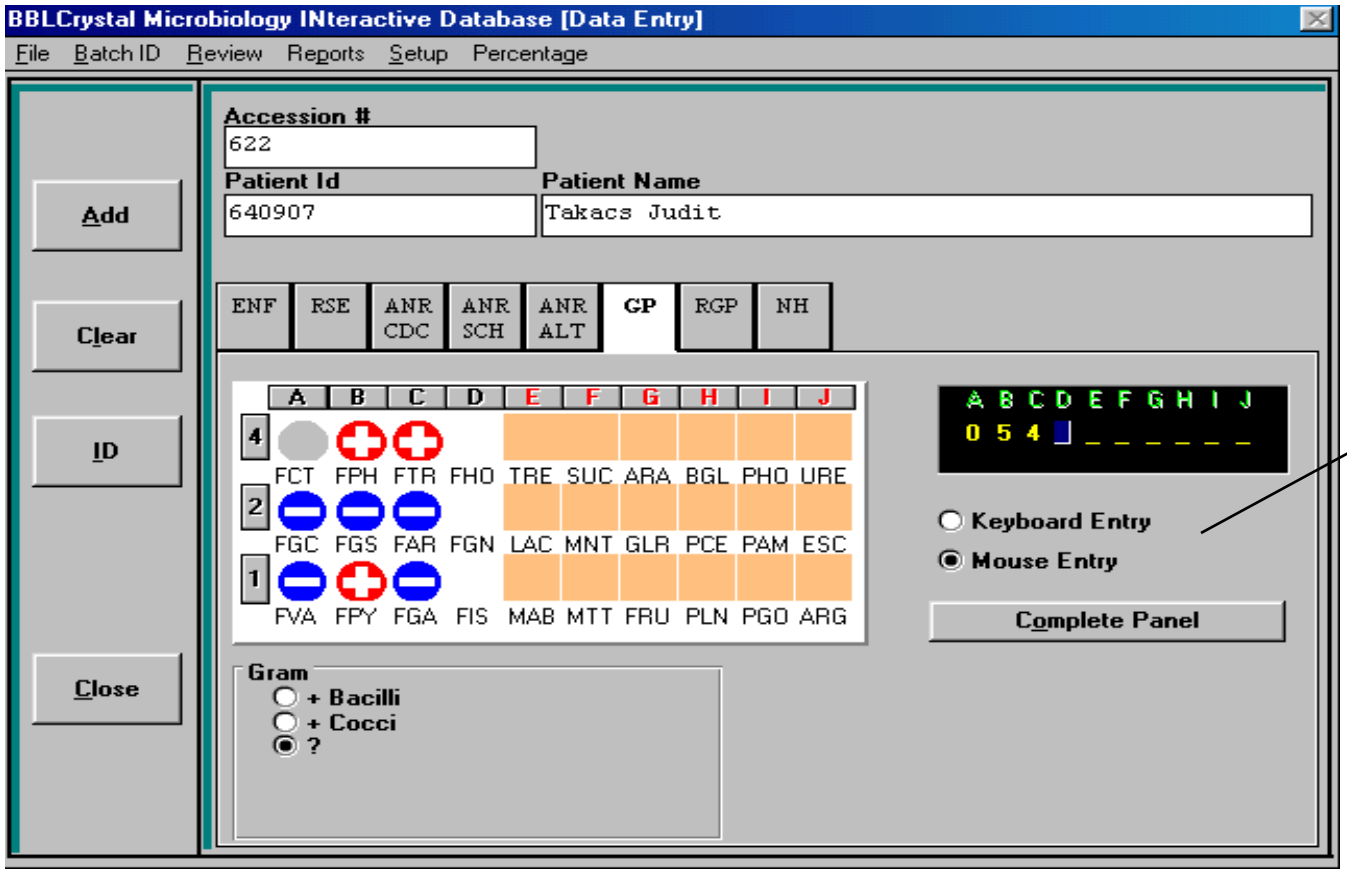
Delete

Profil

Virtual field

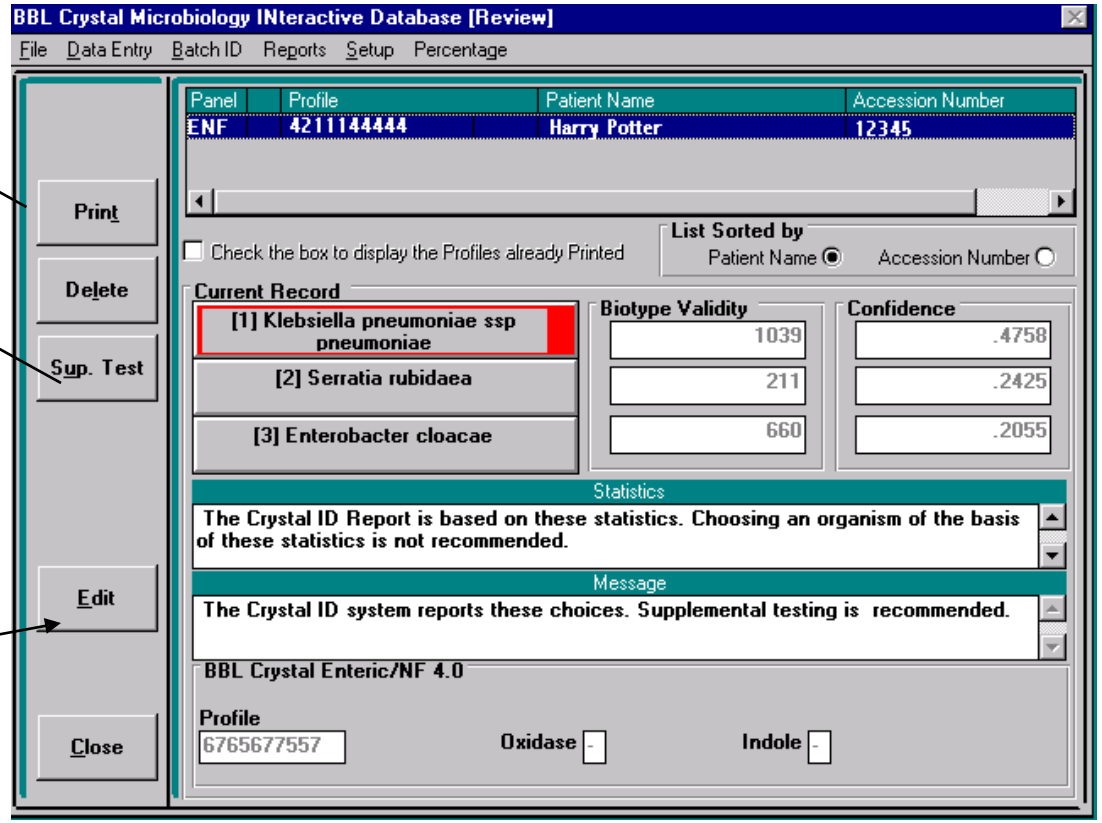
Off-line tests

BBL CRYSTAL MIND



Virtul field and profil are moving together

BBL CRYSTAL MIND



Specimen report

Suppl. tests

Back to data-entry

Worklist

Results-field

Special messages

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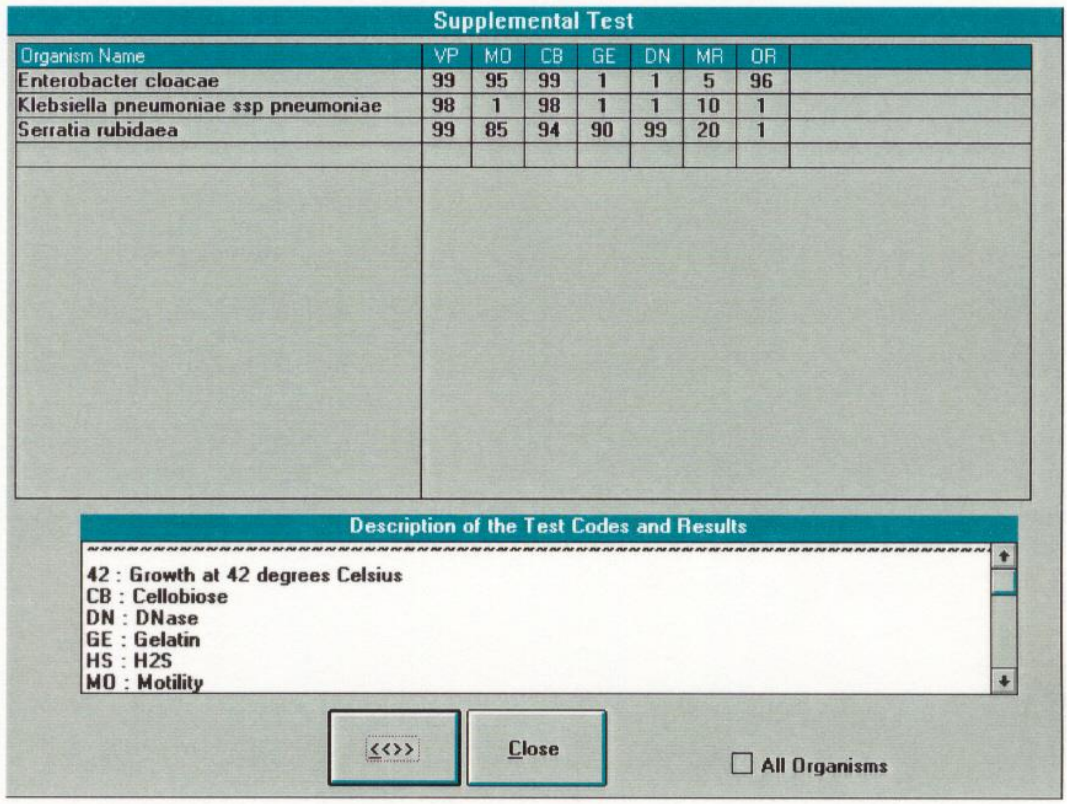
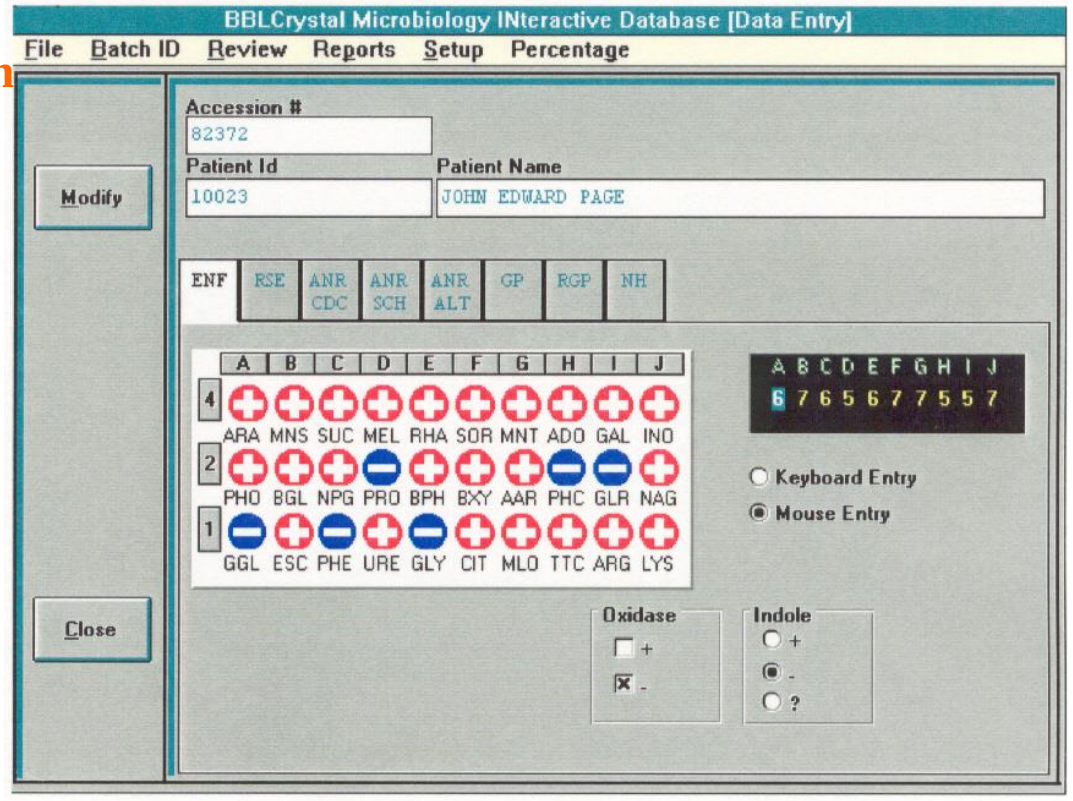


Percentages of positivity

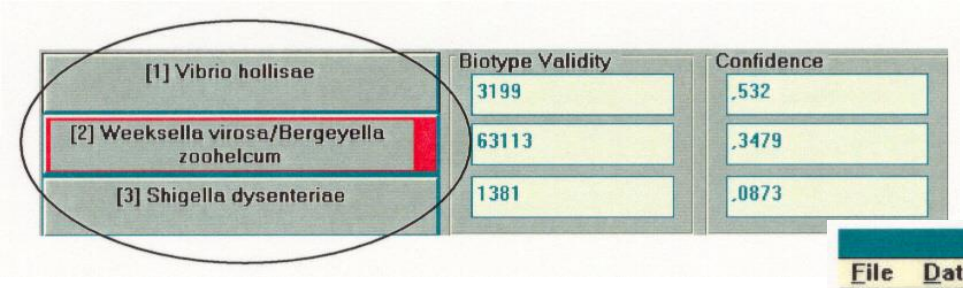
Explanation for abreviations

BBL CRYSTAL MIND

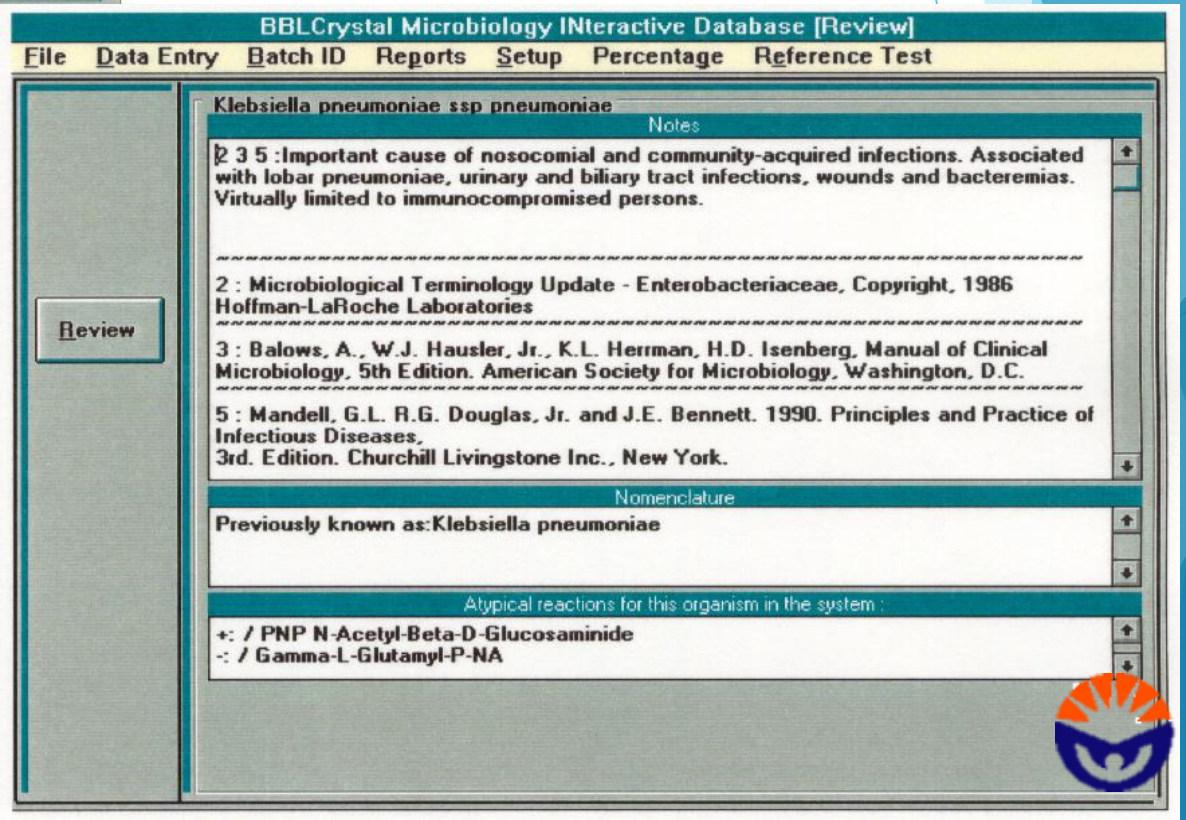
Modification of results



BBL CRYSTAL MIND



Organism information



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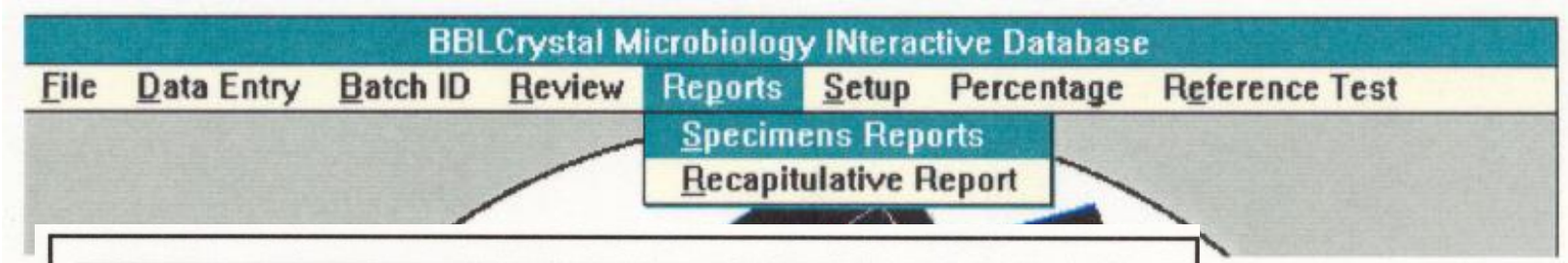

BBL Crystal MIND - Specimen Report

4/30/1999 11:48:06

Accession #: 82372
Patient Id: 10023
Patient Name: JOHNEDWARDPAGE
Profile: 6765677557 *BBL CrystalEnteric/NF 4.0*

Indole: -
Oxidase: -

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

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

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

	NI	WP	MD	NV	GB	GE	CN	MR	PR	HS	OB	M2
KLEPNEP	99	98	1	99	98	1	1	98	?	1	1	?
SERRUB	99	99	85	99	94	99	99	20	?	1	1	?
ENTCLO	99	99	95	99	99	1	1	5	?	1	96	?
Rarity												

Final Id :

Atypical Reactions:

	ARA	PHO	GGL	MNS	BGL	ESC	SUC	NPG	PHE	MEL	PRO	URE	RHA	BPH	GLY	SOR	BXY	CIT	MNT	AAR	MLO	ADO	PHC	TTC	GAL	GLR	ARG	NO	NAG	LYS
KLEPNEP			-																										+	
SERRUB			-										+							+										
ENTCLO			-																									+		

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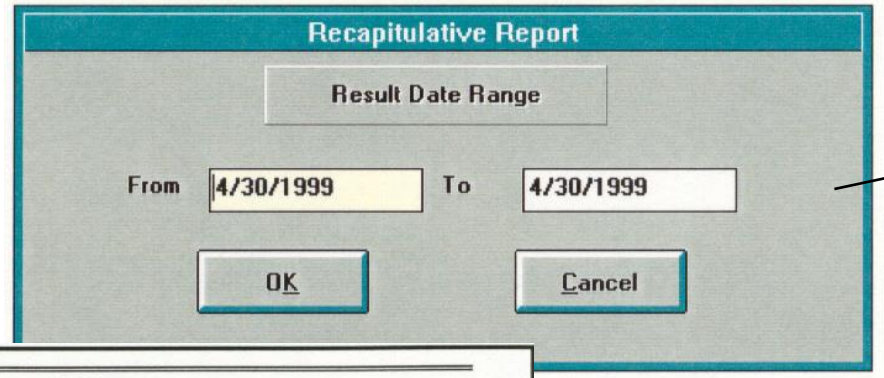
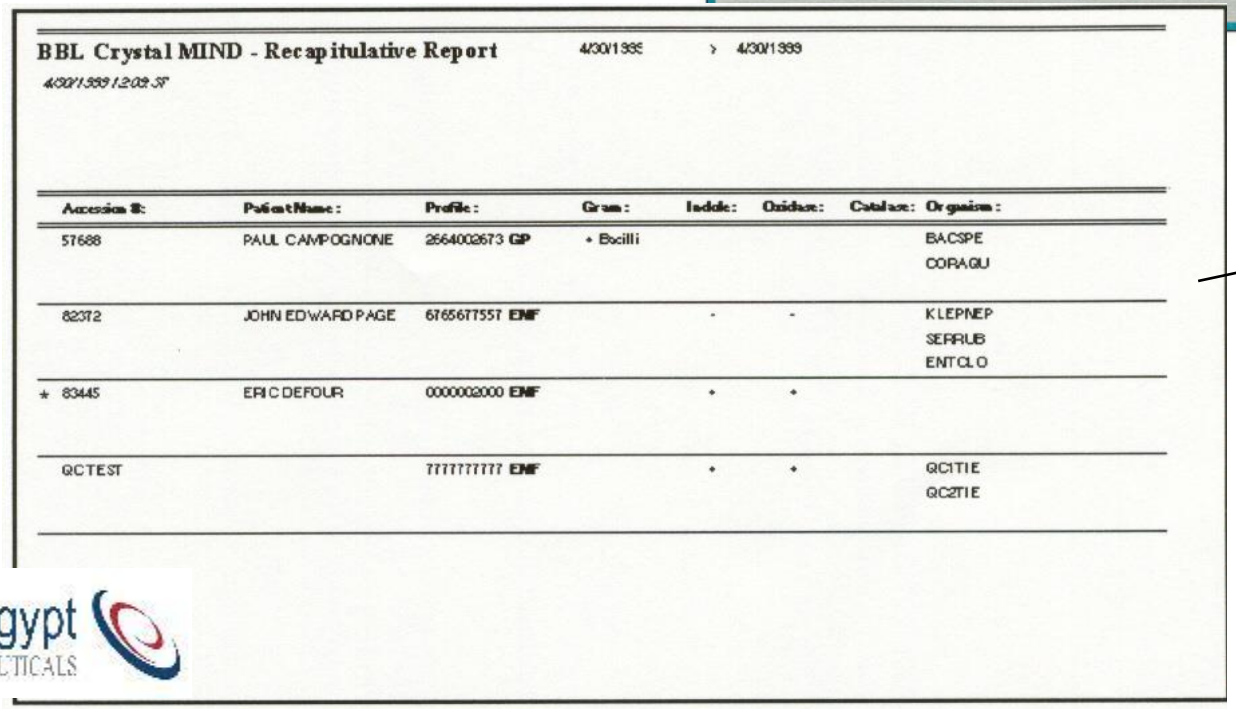


Giving of data range



List of results within the given range of time

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THANK YOU

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Dr. Emad Reda