IDENTIFICATION OF *CORYNEBACTERIUM* (API CORYNE)

Principle

The API CORYNE system facilitates the 24 hour identification of *C. jeikeium* (CDC Group JK), other medically important Corynebacteria, *Rhodococcus equi*, *Listeria* species, *Erysipelothrix rhusiopathiae*, Actinomyces pyogenes, Arcanobacterium haemolyticum, *Brevibacterium* species and *Gardnerella vaginalis*.

The API CORYNE strip consists of 20 microtubes containing dehydrated substrates for the demonstration of enzymatic activity or the fermentation of carbohydrates (CHO). The addition of a dense test suspension of bacteria rehydrates the enzymatic substrates. The metabolic end products produced during incubation are detected through spontaneous coloured reactions or by the addition of reagents.

The fermentation tests are inoculated with an enrichment medium (containing pH indicator) which reconstitutes the CHO substrates. Fermentation of CHO is detected by colour change in the pH indicator.

Materials

API Coryne strips - store at 2 - 8^0C
GP medium - store at 2 - 8^0C
McFarland Standard #6
Nitrate A - store at Room Temperature
Nitrate B - store at 2 - 8^0C
Zym A - store at 2 - 8^0C in the dark
Zym B - store at 2 - 8^0C in the dark
PYZ - store at 2 - 8^0C in the dark
H_2O_2 - store at 2 - 8^0C
Mineral oil
Sterile saline 3 ml

Procedure

1. Preparation of Inoculum

   a) Only pure cultures of a single organism should be used (heavily inoculated sheep BAP x 3; incubate for 24 hours at 35^0C in 5% CO_2).

   b) Using a sterile swab, harvest all the culture from 3 BAP and inoculate into 3 ml. sterile saline to give a turbidity of at least McFarland #6.

2. Preparation of the Strip

   a) An incubation tray is supplied for each strip.

   b) Dispense 5 ml of water into the wells of the tray.

3. Inoculation of the Strip

   a) Inoculate tests 1 $\rightarrow$ 11 of the strip (NIT to GEL).

   b) Avoid bubbles by tilting the strip slightly forward while placing the pipette tip on the side of the cupule.

   c) Add 3 drops into each cupule for tests NIT to ES.

   d) For the URE test fill the tube portion only.

   e) For the GEL test, fill both the tube and cupule. Then:

   f) For the last nine tests of the strip (O to GLYG transfer the rest of the bacterial suspension to an ampoule of GP medium. Mix well.

   g) Distribute the new suspension into the tubes only of tests O to GLYG

   h) Overlay cupules URE and O to GLYG with mineral oil, forming a slight convex meniscus.

   i) Cover with incubation lid and incubate the strip for 24 hours at 35^0C (non-CO_2).

Interpretation

REACTIONS TABLE

TESTS	REACTONS	NEGATIVE RESULTS	POSITIVE RESULTS
NIT	NITrate reduction	**NIT A + NIT B**	(10 mn)
		Colourless Very pale pink	Dark pink Red
PYZ	PYraZinamidase	**PYZ**	(10 mn)
		Colourless Very pale brown Very pale orange	Brown Orange
PyrA	Pyrrolidonyl Arylamidase	**ZYM A**	**+ZYM B**
		PyrA → B	NAG (10 mn)
		Colourless Pale orange	Orange
PAL	Alkaline Phosphatase	Colourless Beige-pale purple Pale orange	Purple
βGUR	Beta GlucURonidase	Colourless Pale grey Pale beige	Blue
βGAL	Beta GALactosidase	Colourless Beige-pale purple	Purple
∝ GLU	Alpha GLUcosidase	Colourless Beige-pale purple Pale green	Purple
BNAG	N-Acetyl-B Glucosaminidase	Colourless Beige-pale purple Pale brown Pale grey	Brown

REACTIONS TABLE (Cont'd)

TESTS	REACTONS	NEGATIVE RESULTS	POSITIVE RESULTS
ESC	ESCulin (β Glucosidase)	Colourless Grey	Black
URE	UREase	Yellow Orange	Red Pink
[GEL]	GELatine (hydrolysis)	No diffusion of black pigment	Diffusion of black pigment
O	**Control (Fermentation)**		
GLU	GLUcose }		
RIB	RIBose }		
XYL	XYLOSE }		
MAN	MANnitol } Fermentation		
MAL	MALtose }	Red	Yellow
LAC	LACtose }		
SAC	Sucrose }	Orange	Yellow-orange
GLYG	GLYcoGen }		
CAT	CATalase (ESC or GEL test)	H_2O_2 3%	1 min
		No bubbles	Bubbles

References

1. Coyle, Marie B., Benjamin Lipsky. 1990. Coryneform Bacteria in Infectious Diseases: Clinical and Laboratory Aspects. Clinical Microbiology Reviews. 3:227-246.

2. Freney, J.M.T. Duperron, C. Couturier, W. Hansen, F. Allard, J.M. Boueufgras, D. Monget, J. Fleurette. Evaluation of API Coryne in Comparison with Conventional Methods for Identifying Coryneform Bacteria, Journal of Clinical Microbiology, January 1991, Vol. 29, p. 38-41.

3. Murray P.A., et al. Manual of Clinical Microbiology, 7th ed., 1999.