API Coryne

I. PRINCIPLE

API Coryne is a miniaturized system, which facilitates the 24-hour identification of medically important coryneform bacteria. It consists of microcupules containing dehydrated substrates for the demonstration of enzymatic activity or the fermentation of carbohydrates. The strip contains reagents for determination of the following biochemical tests: nitrate reduction, pyrazinamidase, pyrrolidonyl arylamidase, alkaline phosphatase, β-glucuronidase, β-galactosidase, α- glucosidase, N-acetyl-β-glucosaminidase, esculin, urease, gelatin hydrolysis, fermentation control, glucose, ribose, xylose, mannitol, lactose, sucrose and glycogen.

II. MATERIALS AND REAGENTS

A. API Coryne strips (store at 2-8 °C until stated expiration date)
B. Suspension media (3.0 mL of sterile distilled water), store at 2-8 °C
C. GP medium (saline with cystine, tryptone, and phenol red), store at 2-8 °C
D. #6 McFarland ($BaSO_4$) standard
E. Incubation tray
F. Nitrate A (Sulfanilic Acid). Store at 2-8 °C until stated expiration date
G. Nitrate B (N-N-Dimethyl-α-naphthylamine). Store at 2-8 °C until stated expiration date
H. Zym A = Tri-hydroxy-methyl-amino-methane, store at room temperature
    1. Attach reactivity sticker labeled: 2 = health, 0 = flammability, 1 = reactivity
I. Zym B = Fast blue BB (sensitive to light, wrap bottle in aluminum foil, store at 2-8 °C)
    1. Attach reactivity sticker: 2 = health, 3 = flammability, 0 = reactivity
J. PYZ = $FeCl_2$ (sensitive to air, keep bottle tightly closed, store at 2-8 °C)
    1. Attach reactivity sticker labeled: 2 = health, 2 = flammability, 0 = reactivity
K. 3% hydrogen peroxide
L. Mineral oil
M. Disposable plastic pipets
N. 37 °C, non-CO_2 incubator
O. Sterile swabs

III. QUALITY CONTROL

Each lot number of API Coryne strips must be tested for expected results. The recommended reference strains are:

1. *Microbacterium testareum* ATCC 15829
2. *Corynebacterium renale* ATCC 19412
3. *Celluosimicrobium cellulans* ATCC 27402
4. *Listeria grayi* ATCC 25401

	NIT	PYZ	PyrA	PAL	BGUR	BGAL	aGLU	BNAG	ESC	URE	GEL	0	GLU	RIB	XYL	MAN	MAL	LAC	SAC	GLYG	CAT
1	-	+	-	-	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	+
2	+	+	+	+	-	+	+	+	+	-	V	-	+	+	+	-	+	-	+	V	+
3	-	+	-	V	-	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	+
4	V	V	-	-	-	-	V	+	+	-	-	-	+	+	-	+	+	+	-	-	+

IV. Safety

A. Do not splash reagents. They may cause irritation to the skin or eyes. Rinse affected areas with water upon exposure.

V. PROCEDURE

A. The organism to be identified must be subcultured to Columbia CNA or TSA agar with 5% sheep blood prior to testing.

B. Only pure cultures of a single organism can be tested.

C. Use a sterile swab to make a suspension equivalent to at least a #6 McFarland standard in an ampule of suspension medium. Compare it to the turbidity control included in the kit.

D. Obtain an incubation tray and lid, and label with the appropriate patient information and the date. Dispense 5 mL of tap water into the tray to provide a humid atmosphere during incubation.

E. Tilt the tray and inoculate tests one through eleven by using a disposable plastic pipet filled with standardized inoculum. Inoculate tests NIT to ESC with 3 drops only. For URE fill the tube only. For GEL fill to the top. For the last nine tests (0 to GLYG), transfer 0.5 mL (9 drops) of the standardized inoculum into an ampule of GP medium to dilute the inoculum, mix well and inoculate the tubes only.

F. Overlay URE and 0 to GLYG with mineral oil.

G. Cover the tray with the lid and incubate for 24 hours at 35 °C in a non-CO_2 incubator.

H. After incubation, add the appropriate reagents as noted below and wait 10 minutes for reactions to occur.

1. Nit test: 1 drop of NIT A and 1 drop of NIT B
2. PYZ test: 1 drop of PYZ
3. Pyr A, PAL, βGUR, βGAL, αGLU, and βNAG tests: 1 drop of ZYM A and ZYM B
4. Perform the catalase test by adding 1 drop of 3% hydrogen peroxide to the ESC or GEL tube. Wait 1 minute for the appearance of bubbles indicating a positive test.

I. Refer to the API Coryne reactions (Table 1) to determine reactions and record on the result sheet.

VI. INTERPRETATION

A. After recording the reactions on the report sheet, a 7-digit number is obtained.

B. Access online database at: https://apiweb.biomerieux.com/jsp/ident/index.jsp

1. Enter Login: **JSHMICRO** and Password: **SHMICRO**
2. Select API CORYNE from list of tests.
3. Enter reactions and click Confirm to see results.

B. If the organism does not identify, bring to the attention of the supervisor.

Table 1 (Reading Table)

Tests	Substrates	Enzymatic Activity or Reaction Tested	Negative	Positive
NIT	Potassium Nitrate	Reduction of Nitrates	NIT 1 + NIT 2 / 10min colorless or pale pink	dark pink or red
PYZ	Pyrazinecarboxamide	Pyrazinamidase	PYZ / 10 min colorless very pale: brown or orange	brown orange
PYRA	pyroglutamic acid-B-naphthylamide	Pyrrolidonyl Arylamidase	ZYM A + ZYM B (PYRA -->*B* NAG) / 10 min colorless pale orange	orange
PAL	2-naphthyl-phosphate	Alkaline Phosphatase	colorless beige - pale purple pale orange	purple
βGUR	Naphthol ASBI-glucuronic acid	*B*-Glucuronidase	colorless pale: grey or beige	blue
βGAL	2-naphyl-*B* D-galactopyranoside	*B*-Galactosidase	colorless beige - pale purple	purple
αGLU	2-naphthyl-*a* D-glucopyranoside	*a*-Glucosidase	colorless beige - pale purple pale green	purple
βNAG	1-naphthyl-N-acetyl-*B* D-glucosamide	N-Acetyl-*B*-Glucosaminidase	colorless beige - pale purple pale: brown or grey	brown
ESC	Esculin, ferric citrate	*B*-glucosidase	colorless or grey	black
URE	Urea	Urease	yellow or orange	red or pink
GEL	Gelatin	Hydrolysis	no diffusion of pigment	diffusion of blk pigment
0	Negative control	Fermentation		
GLU	D-glucose	Fermentation		
RIB	D-ribose	Fermentation		
XYL	D-xylose	Fermentation	red	yellow
MAN	D-mannitol	Fermentation	orange	yellow-orange
MAL	D-maltose	Fermentation		
LAC	D-lactose	Fermentation		
SAC	D-saccharose (sucrose)	Fermentation		
GLYG	glycogen	Fermentation		
CAT	(ESC or GEL test)	Catalase	H_2O_2 (3%) / 1 min no bubbles	bubbles

VII. Reference

A. Gavin, S., R. Leonard, A. Briselden and M. Coyle. 1992. Evaluation of the Rapid Coryne identification system for *Corynebacterium* species and other coryneforms. J Clin Microbiol 30:1692-1695.

B. Package Insert: Biomerieux API CORYNE 07886F 2006/01

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Updates and Revisions: