Instruction for Use for Diatabs™

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Diatabs™
For bacterial identification

Manufacturer
ROSCO Diagnostica A/S, Taastrupgaardsvej 30, DK-2630 Taastrup, Denmark, www.rosco.dk

Intended use
To be used in qualitative procedures to detect *in vitro* microbial properties for the identification of microorganisms. The identification tests are available as individual tablets.

Principles of the procedure
Diatabs can be divided into two groups: Diatabs to be placed on agar plates, and Diatabs to be dissolved in liquid. Most of the Diatabs provide rapid tests using chromogenic enzymatic reactions, and modified conventional tests. Diatabs placed on agar plates detect natural susceptibility patterns or growth factor requirements. Following incubation, the plates are examined and the zone diameters of inhibition around the tablet are measured and compared with the zone interpretation table (sensitive/resistant) for the individual tablets. Diatabs used in liquid are based upon enzymatic properties of the microorganisms detected by various indicator systems. [1]

More information about Diatabs for bacterial identification may be obtained from User's Guide Diatabs [2] at www.rosco.dk.

Storage instructions

1) On receipt check the temperature symbol on the label. Diatabs with a 2 °C to 8 °C symbol should be stored in a refrigerator, and Diatabs with a 25 °C as an upper temperature symbol on the label should be stored at room temperature.
2) If Diatabs are stored in the refrigerator, allow the vials to reach room temperature before opening, i.e. 30 - 60 minutes, in order to avoid condensation forming on the tablets.
3) Keep Diatabs in vials well protected from direct light and avoid high humidity. Keep, if any, the humidity absorbing material (a desiccant capsule) in the vial.

The expiry date on the vials applies only to vials with lids stored at the correct temperature.

Reagents
Diatabs are 6 or 9 mm tablets furnished in vials each containing 15, 25, or 50 tablets. The 9 mm tablets are print-coded on both sides with a unique 5-character code. The 6 mm tablets used on agar are colour-coded and the Diatabs to be dissolved in liquid have no code. The user should keep track of the contents of the test tube when using more than one Diatabs.

Precautions
Follow the directions for use. None of the tablets are intended for susceptibility testing. Diatabs performance depends not only on the specific Diatabs but also on use of proper inoculum, incubation time and interpretation of zone diameter or colour change. Only properly trained technicians should use the products. Use a pair of tweezers to pick up the Diatabs.
Precautions should be taken against microbiological hazards by properly sterilizing specimens, containers, media, and test tubes after use. Diatabs, test tube and agar plates with potential pathogens are disposed of as microbiological waste.

Specimen
The specimen should be collected and handled following recommended guidelines. The specimen should be a pure culture fully typical of the species to be identified.

Procedure

Materials provided: Diatabs as labelled on the container.

Materials required but not provided: Culture media, test tubes, reagents, quality control organisms and laboratory equipment necessary to perform identification e.g. inoculating loops, swabs, pipettes and collection containers.

I. Diatabs placed on agar

The susceptibility of microorganisms isolated from clinical samples against antimicrobials and other agents may be useful in the identification. It is possible to characterize and distinguish microorganisms by measuring the size of the zone diameter after culturing with a standardized inoculum applied to a specific agar medium.

I.1. Inoculum Preparation
Suspend several morphologically similar colonies from an 18-24 h cultured agar plate (non-selective) into 4-5 ml 0.9 % NaCl solution to obtain turbidity comparable to 0.5 McFarland standard.

I.2. Inoculation
Within 15 minutes, dip a sterile cotton swab into the adjusted suspension and remove inoculum from the swab by exerting firm pressure on the inside of the tube. Within 15 minutes swabs are used to inoculate the agar specified in Table 1. Inoculate the dried surface of the appropriate agar plate by streaking the swab over the entire surface. Allow the surface to dry for 3-5 minutes or maximum 15 minutes before applying Diatabs to the agar surface.

I.3. Incubation and reading of plates
Within 15 minutes, place the agar side up and incubate plates depending on species and according to Table 1 (aerobic, anaerobic, or in 5-10% CO_2). Examine the plates after overnight incubation, if nothing else is specified in Table 1. The diameters of the zones of complete inhibition are measured as determined by gross visual inspection. Zones are measured to the nearest whole millimetre.

RESULTS:
Compare recorded zone diameter with those in Table 1. Results for a specific specimen may be reported as Sensitive (S) or Resistant (R) according to the interpretation zones. Use Table 1 to make a preliminary identification of the bacterial strain.

II. Diatabs used in liquid

II.1. Inoculum preparation
The organism to be tested should be 18-24 hours old and in pure culture. Slow-growing isolates may be tested using 48-hour old culture.

Prepare a heavy suspension (at least McFarland 4 standard) of the test organism in 0.25 ml saline in a tube. A battery of Diatabs may be inoculated with a single inoculum.

II.2. Inoculation
Add one Diatabs to the tube. Some Diatabs additionally require 3 drops of sterile paraffin oil added to the tube (specified in Table 2).

II.3. Incubation and reading of tubes
Seal the tube and incubate at 35-37 °C for 4 hours, overnight or as specified in Table 2.
After incubation observe for colour development. For some Diatabs a reagent has to be added before reading the colour and some Diatabs may demonstrate two reactions.
After interpretation of colour development, add a reagent and read the new colour (specified in Table 2).

RESULTS:
Diatabs in liquid are scored by colour reactions. Table 2 gives colour reactions for negative and positive strains. Record the test score in an appropriate report form.

III. Diatabs with a different procedure

Oxidase
Lay a thick filter paper in an empty petri dish and place the Diatabs on it. Add one drop of saline on top of tablet, wait 60 seconds and add another drop of saline on the top. When the filter paper is wet, smear the colony onto the wet filter paper approximately 3-8 mm from the edge of the tablet using a plastic loop. Reading after 2 minutes. More than one isolate can be tested using the same Diatabs and filter paper.

Factor X, V, and X+V
Tablets with growth factors, hemin (X-Factor) and NAD (V-Factor) or both factors are used for the differentiation of the *Haemophilus* spp. All three tablets are placed onto the agar, Factor X and Factor V at a distance of 2 cm from each other and factor X+V further away from these. Use only medium free of the two growth factors (e.g. TSA agar). Incubate overnight at 35-37 °C for 18-24 hours, and observe for growth or no growth in the vicinity of a tablet. If the organism requires Factor X alone, it will grow only in the vicinity of the X and X + V factor tablets; if it requires Factor V alone, it will grow only in the vicinity of the V and the X + V factor tablets; if both X and V factors are required, it will grow only in the vicinity of the X + V factor tablets.[1]

INTERNAL QUALITY CONTROL
Quality control procedure using ATCC strains or known positive or negative strains should be used to monitor the performance of the tablets. Quality control should be performed in accordance with established laboratory quality control procedures. Negative/positive colour reactions or sensitive/resistant strains are given in the Tables and indicate correct performance of the entire procedure. If aberrant quality results are noted, the results should not be reported.

LIMITATIONS OF METHOD

1. Pure cultures of microorganisms should be used, since a mixed microbial population will result in aberrant results.
2. Use of Diatabs for the identification of microorganisms and interpretation of results require a technician trained in general microbiological methods and who should judiciously make use of knowledge, experience, specimen information, and other pertinent procedures before reporting identity of the isolate.
3. Results from additional tests may affect the final identification result.
4. The tablets are not intended for use in susceptibility testing procedures.
5. The accuracy of the Diatabs is based upon known *in vitro* microbial properties for specific clinically important bacterial species [1]. If atypical or inconsistent results are encountered, repeat testing is recommended. An unexpected result should be considered for reporting and isolates could be sent to reference laboratories for further testing.

REFERENCES

1) Murray P.R. et al. 2003. Manual of Clinical Microbiology, 8th ed., ASM, Washington DC.
2) Diatabs User's Guide 5th ed. 2000. www.rosco.dk

Table 1. Interpretation of results from Diatabs (on agar)

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DIATABS (Active ingredients per tablet)	Size mm	Code*	Procedure	Interpretation				QC strain	
				Zone mm	Sensitive	Zone mm	Resistant	Sensitive	Resistant
Bacitracin low 0.4 U	6	BaL	Standard I on TSA blood agar	> 15	Beta-hæmolytic streptococci Group A	< 14	Other streptococci	*S. pyogenes* ATCC 12344	*S. bovis* ATCC 15351
Boronic Acid 250 µg (Research use only)	9	**BORON**		Detection of plasmid mediated AmpC beta-lactamases.					
Brilliant Green 100 µg	6	BrG	Standard II	≥ 10	*Bacteroides* spp.	< 10	*Fusobacterium* spp.	*B. fragilis* ATCC 25285	*F. necrogenes* ATCC 25556
C-390 40 µg	9	**C-390**	Standard I	≥ 12	Other *Pseudomonas* spp. and non-fermenters	No zone	*P. aeruginosa*	*S. maltophilia* ATCC 13637	*P. aeruginosa* ATCC 27853
Colistin 10 µg (Colistin sulphate)	9	**Co.10**	Standard II	≥ 10	E.g. *Fusobacterium* spp. [d]	No zone	E.g. *B. fragilis* group [d]	*E. coli* ATCC 25922	*S. aureus* ATCC 25923
Cloxacillin 500 µg (Research use only)	9	**CL500**		Detection of plasmid mediated AmpC beta-lactamases.					
Cycloheximide 15 µg (Cycloheximide < 0.02 %)	9	**CYC15**	Standard I on modified Shadomy agar	≥ 25	*C. glabrata* *C. krusei* *C. lusitaniae*	< 25	*C. albicans* *C. pseudotropicalis*	*C. albicans* ATCC 64548	*C. krusei* ATCC 6258
Deferoxamine 250 µg (Deferoxamine mesylate)	9	**DEFRX**	Standard I [a]	≥ 16	*S. epidermidis* *S. hominis* *S. lutrae*	≤ 14	Other staphylococci	*S. epidermidis* ATCC 12228	*S. aureus* ATCC 25923
Factor V (b-Nicotinamide adenine dinucleotide sodium 175 µg)	6		See text		*H. influenzae*: Both X-Factor and V- Factor required. Growth around Factor X+V and between the Factor X and Factor V. *H. parainfluenzae*: Only V- Factor required. Growth around Factor V and Factor X+V.			*H. influenzae* ATCC 49247 *H. parainfluenzae* ATCC 7901	
Factor X (Hemin chloride 260 µg)	6								
Factor X + V	6								
Metronidazole 5 µg	9	**MTR.5**	Standard II	≥ 15	Anaerobic bacteria	No zone	Aerobes and micro-aerophilic bacteria	*B. fragilis* ATCC 25285	*E. coli* ATCC 25922
Metronidazole 50 µg	9	**MTR50**	Standard III	≥ 12	*Gardnerella vaginalis*	< 12	Lactobacilli Coryneoforms	*G. vaginalis* ATCC 14018	*E. coli* ATCC 25922

DIATABS (Active ingredients per tablet)	Size mm	Code*	Procedure	Interpretation				QC strain	
				Zone mm	Sensitive	Zone mm	Resistant	Sensitive	Resistant
O/129 150 µg (2,4-Diamino-6,7-diisopropyl-pteridine phosphate salt)	9	**O/129**	Standard [b]	≥ 16	*Vibrio* spp. Plesiomonas	< 16	Aeromonas Enterobacteriaceae	*Kocuria rhizophila* ATCC 9341	*E. coli* ATCC 25922
Optochin 10 µg (Ethylhydrocuprein HCl)	6	OPT	Standard TSA with 5 % blood. Incubation with or without CO_2	≥ 18 (CO_2) ≥ 20 (aerob)	*S. pneumoniae*	< 16 (CO_2) < 18 (aerob)	Other non-haemolytic streptococci	*S. pneumoniae* ATCC 49619	*S. bovis* ATCC 15351
Oxgall 1000 µg (Oxgall)	6	OXG	Standard II	> 9	E.g. *Porphyromonas* spp. [d]	No zone	E.g. *Bacteroides fragilis* group [d]	*S. aureus* ATCC 25923	*B. fragilis* ATCC 25285
Ps. aeruginosa Screen 80 µg (1,10 Phenanthroline)	9	**PSAE**	Standard I [c]	≥ 18	Other *Pseudomonas* spp., non-fermenters, and Enterobacteriaceae	≤ 14	*P. aeruginosa*	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
S.P.S. 1000 µg (Sodium polyanethol sulfonate)	6	SPS	Standard III	≥ 10	*Gardnerella vaginalis*			*G. vaginalis* ATCC 14018	*Kocuria rhizophila* ATCC 9341
Tellur 500 µg (Potassium tellurite)	6	TEL	Standard I	> 15	Streptococci and most other enterococci	< 12	*E. faecalis*	*S. bovis* ATCC 15351	*E. faecalis* ATCC 29212

* Code in bold is printed on the tablets.

Procedure:

Standard I: The test is performed with an inoculum equivalent to McFarland 0.5 on Mueller Hinton Agar incubated aerobically at 35-37 °C overnight if agar or incubation condition is not specified in the table.

Standard II: The test is performed with an inoculum equivalent to McFarland 0.5 on FAA+blood incubated anaerobically at 35-37 °C for 24-48 hours if agar or incubation condition is not specified in the table.

Standard III: The test is performed with an inoculum equivalent to McFarland 0.5 on Mueller-Hinton Agar + blood incubated at 35-37 °C with 5-10 % CO_2 overnight if agar or incubation condition is not specified in the table.

a) Measure the zone up to colonies of normal size, disregard semiinhibited colonies. Do not use agar with blood.
b) Use Oxoid Blood Agar Base with 0.5% NaCl. Incubation for 24 hours. Acquired resistance against trimethoprim will also be resistant to O/129.
c) Measure only the clear zone.
d) Can be used for separating the major groups of common anaerobic bacteria.

Table 2. Interpretation of results from Diatabs (used in liquid)

DIATABS (Active ingredients per tablet)	Code	Procedure	Incubation time	Interpretation		Quality Control	
				Positive	Negative	Positive	Negative
Acetamide Hydrolysis (Acetamide 1.5 mg)	ACM	Standard	(4 h) or overnight	Red	Yellow, orange	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922
Adonitol (Adonitol 3 mg)	ADO	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*K. pneumoniae* ATCC 13883	*E. coli* ATCC 25922
Alkaline Phosphatase (p-Nitrophenyl-Phosphate 0.1 mg)	Alk P	Standard	4 h	Strong yellow	Colourless, light yellow	*E. coli* ATCC 25922	*S. haemolyticus* ATCC 29970
Alpha-Fucosidase (p-Nitrophenyl-α-L-Fucosidase 0.2 mg)	α-FUC	Standard	4 h or overnight	Yellow	Colourless	*B. fragilis* ATCC 25285	*E. coli* ATCC 25922
Alpha-Galactosidase (p-Nitrophenyl- α-D-Galactopyranoside 0.3 mg)	α-GAL	Standard	4 h or overnight	Yellow	Colourless	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
Alpha-Glucosidase (p-Nitrophenyl-α-D-Glucopyranoside 0.3 mg)	α-GLU	Standard	4 h or overnight	Yellow	Colourless	*S. maltophilia* ATCC 13637	*P. aeruginosa* ATCC 27853
Alpha-Mannosidase (p-Nitrophenyl-α-Mannopyranoside 0.3 mg)	α-MAN	Standard	4 h or overnight	Yellow	Colourless	*L. monocytogenes* ATCC 19115	*E. coli* ATCC 25922
Arginine Dihydrolase (L-Arginine HCl 3 mg)	ADH	Standard+oil	4 h or overnight	Red	Yellow	*P. aeruginosa* ATCC 27853	*K. pneumoniae* ATCC 13883
Beta-Fucosidase (p-Nitrophenyl-ß-L-Fusosidase 0.2 mg)	ß-FUC	Standard	4 h or overnight	Yellow	Colourless	*S. intermedius* ATCC 27335	*E. coli* ATCC 25922
Beta-Glucosidase (p-Nitrophenyl-ß-D-Glucopyranoside 0.3 mg)	β-GLU	Standard	4 h or overnight	Yellow	Colourless	*K. pneumoniae* ATCC 13883	*Morganella morganii* ATCC 25830
Beta-Glucuronidase (PGUA) (p-Nitrophenyl- ß -D glucuronic acid 0.3 mg)	PGUA	Standard	4 h or overnight	Yellow	Colourless	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 13883
Beta-Lactamase (Acido) (Penicillinprocaine 4 mg, Penicillin G sodium 4 mg)		Standard	15-20 min and up to 4 h	Yellow (or brownish)	Violet	*S. aureus* ATCC 29213	*S. aureus* ATCC 25923
Beta-N-Acetylglucosaminidase (p-Nitrophenyl-N-acetyl-β-D-glucosaminide 0.2 mg)	β-NAG	Standard	4 h or overnight	Strong yellow	Colourless	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 25923
Beta-Xylosidase (p-Nitrophenyl ß-D-xylopyranoside 0.3 mg)	β-XYL	Standard	4 h or overnight	Yellow	Colourless	*K. pneumoniae* ATCC 13883	*E. coli* ATCC 25922
Bile Esculin (Esculin 2 mg, Oxgall 9 mg)	BE	Standard	4 h or overnight	Black, grey	Colourless, light grey	*E. faecalis* ATCC 29212	*S. pneumoniae* ATCC 49619
Citrate (Citrate 2 mg)	CIT	Standard	18-24 h	Red	Yellow, orange	*P. aeruginosa* ATCC 27853	*Proteus vulgaris* ATCC 13315
d-Xylose (d-Xylose 2 mg)	XYL	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*E. coli* ATCC 25922	*Morganella morganii* ATCC 25830

DIATABS (Active ingredients per tablet)	Code	Procedure	Incubation time	Interpretation Positive	Interpretation Negative	Quality Control Positive	Quality Control Negative
Esculin Hydrolysis (Esculin 2 mg)	ESC	Standard	4 h (or up to 24 h)	Black, grey	Colourless, light grey	*K. pneumoniae* ATCC 13883	*E. coli* ATCC 25922
Gamma-Glutamyl Aminopeptidase (Gamma-Glutamyl-β-Naphthylamide 0.2 mg)	γ-GLU	Standard+I	4 h or overnight	Red-orange Red	Yellow Yellow, orange	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922
Glucose (D-Glucose monohydrate 2 mg)	GLU	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*P. aeruginosa* ATCC 27853	*A. lwoffii* ATCC 9957
Hippurate Hydrolysis (Hippuric acid Sodium-salt 3 mg)	HIP	Standard+IV	4 h	Deep purple, blue	Colourless, light yellow	*S. agalactiae* ATCC 12386	*S. pyogenes* ATCC 12344
Indoxyl Acetate (Indoxyl acetate 5 mg)	IAC	Standard	4 h or overnight	Blue, green sediment	Colourless, slightly coloured supernatant	*Campylobacter jejuni* ATCC 33291	*E. faecalis* ATCC 51299
Inulin (Inulin 3 mg)	INU	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*S. mutans* ATCC 25175	*E. coli* ATCC 25922
Lactose (Lactose monohydrate 3 mg)	LAC	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
l-Arabinose (L-Arabinose 3 mg)	ARA	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*E. coli* ATCC 25922	*S. aureus* ATCC 25923
LDC/Indole (L-Lysine 3 mg, L-Tryptophane 0.7 mg)	LDC/ IND	Standard+oil. Read LDC first then+IIa	3-4 h (or up to 24 h)	Blue, violet Red	Yellow, green, grey Yellow	*E. coli* ATCC 25922 (LDC pos., IND pos.)	*Proteus vulgaris* ATCC 13315 (LDC neg., IND pos.) *K. pneumoniae* ATCC 13883 (LDC pos., IND neg.)
Leucine Aminopeptidase (L-Leucine-β-naphthylamide-HCL 20 µg)	LAP	Standard+I	4 h or overnight	Red-orange Red	Yellow Yellow, orange	*S. bovis* ATCC 15351	*Aerococcus viridans* ATCC 700406
l-Rhamnose (L-Rhamnose 3 mg)	RHAM	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*K. pneumoniae* ATCC 13883	*Proteus vulgaris* ATCC 13315
Lysine Decarboxylase (LDC) (L-Lysine 3 mg)	LDC	Standard+oil	4 h or up to 24 h	Blue, violet Strong violet	Yellow, green, grey, light blue	*K. pneumoniae* ATCC 13883	*Enterobacter cloacae* ATCC 13047
Maltose (Maltose monohydrate 3 mg)	MAL	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*E. coli* ATCC 25922	*Morganella morganii* ATCC 25830
Mannitol (D-Mannitol 3 mg)	MAN	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
Mannose (D-Mannose 3 mg)	MSE	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
Melibiose (D-Melibiose 3 mg)	MEL	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
Nitrate Reduction (Sodium Molybdate 40 µg, Potassium nitrate 4 mg)	NO_3	Standard+V	4 h or overnight	Red, pink	Colourless, light pink	*E. coli* ATCC 25922	*S. saprophyticus* ATCC 15305

DIATABS (Active ingredients per tablet)	Code	Procedure	Incubation time	Interpretation Positive	Interpretation Negative	Quality Control Positive	Quality Control Negative
ONPG (Beta-Galactosidase) (o-Nitrophenyl-β-D-Galactopyranoside 0.4 mg)	ONPG	Standard	4 h or overnight	Yellow	Colourless	*E. coli* ATCC 25922	*S. aureus* ATCC 25923
ONPG/PYR (o-Nitrophenyl-β-D-Galactopyranoside 0.4 mg, L-pyrrolidonyl-ß-naphthylamide 0.2 mg)	ONPG/ PYR	Standard Read ONPG first, then+1	3-4 h (for non-fermentors 18-24 h)	Yellow Red	Colourless Yellow, orange	*S. haemolyticus* ATCC 29970 (ONPG neg., PYR pos.)	*Proteus vulgaris* ATCC 13315 (ONPG neg., PYR neg.)
Ornithine Decarboxylase (ODC) (L-Ornithine HCl 3 mg)	ODC	Standard+oil	4 h or up to 24 h	Blue, violet Strong violet	Yellow, green, grey, light blue	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 13883
Oxidase (Tetramethyl-p-Phenylenediamine 0.7 mg)	OXI	See text	Reading after 2 min	Blue, purple	No colour change	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922
PGUA/Indole (p-Nitrophenyl-β-D-Glucoronic acid 0.4 mg, L-Tryptophane 1 mg)	PGUA/ IND	Standard Read PGUA first, then+IIa	3-4 h (or up to 24 h)	Yellow Red	Colourless Yellow	*E. coli* ATCC 25922 (PGUA pos., IND pos.) *Proteus vulgaris* ATCC 13315 (PGUA neg., IND pos.)	*Enterobacter cloacae* ATCC 13047 (PGUA neg., IND neg.)
Porphyrin (d-Ala) (d-Aminolevulinic acid 0.1 mg)	ALA	Standard+IIb	4-6 h or up to 24 h	Red, pink in the lower water phase	Colourless water phase	*H. parainfluenzae* ATCC 7901	*H. influenzae* ATCC 49247
Proline Aminopeptidase (L-proline ß-Naphthylamide-HCl 80 µg)	PRO	Standard+I	4 h or overnight	Red-orange Red	Yellow Yellow, orange	*P. aeruginosa* ATCC 27853	*Cl. perfringens* ATCC 12917
Pyrazinamidase (Pyrazinamide 0.9 mg)	PZA	Standard [a]	4 h or overnight	Orange, red	Colourless, light yellow	*E. coli* ATCC 25922	*S. aureus* ATCC 25923
Pyrrolidonyl Aminopeptidase (L-Pyrrolidonyl-ß-Naphthylamide 0.2 mg)	PYR	Standard+I	4 h or overnight	Red-orange Red	Yellow Yellow, orange	*Enterobacter cloacae* ATCC 13047	*E. coli* ATCC 25922
Raffinose (D-Raffinose pentahydrate 3 mg)	RAF	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*Enterobacter cloacae* ATCC 13047	*E. coli* ATCC 25922
Sorbitol (D-Sorbitol 2mg)	SOR	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
Sucrose (Saccharose 3 mg)	SUC	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*Enterobacter cloacae* ATCC 13047	*Morganella morganii* ATCC 25830
TDA or Indole: Indole (L-Tryptophane 1 mg)	IND	Standard+IIa	4 h or overnight	Red (purple, pink) colour of the surface layer	Yellow	*Proteus vulgaris* ATCC 13315	*K. pneumoniae* ATCC 13883
TDA or Indole: TDA (L-Tryptophane 1 mg)	TDA	Standard+III	4 h or overnight	Brown, red	Yellow, orange	*Proteus vulgaris* ATCC 13315	*K. pneumoniae* ATCC 13883
Tetrathionate Reductase (Tetrathionate 2 mg)	TTR	Standard	4 h or overnight	Yellow	Red-orange	*Proteus vulgaris* ATCC 13315	*E. coli* ATCC 25922

DIATABS (Active ingredients per tablet)	Code	Procedure	Incubation time	Interpretation Positive	Interpretation Negative	Quality Control Positive	Quality Control Negative
Trehalose (D-Trehalose dihydrate 2 mg)	TRE	Standard	4 h or overnight	Yellow, yellow-orange	Red, orange red	*E. coli* ATCC 25922	*Morganella morganii* ATCC 25830
Tributyrin (Tributyrin 2 mg)	TRIB	Standard	4 h (or overnight)	Yellow, yellow orange	Red	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922
Trypsin (Na-Benzoyl-DL-Arginine-ß-Naphthylamide 0.2 mg)	TRYP	Standard+I	4 h or overnight	Red-orange Red	Yellow Yellow, orange	*S. maltophilia* ATCC 13637	*E. coli* ATCC 25922
Urease (Urea 8 mg)	URE	Standard	4 h (or overnight)	Red/purple [b]	Yellow, orange	*Proteus vulgaris* ATCC 13315	*E. coli* ATCC 25922
Urease/Indole (Urea 4 mg, L-Tryptophane 1 mg)	URE/ IND	Standard Read URE first, then +IIa	4 h (or overnight)	Red, purple Red	Yellow Yellow, orange	*Morganella morganii* ATCC 25830 (URE pos., IND pos.) *K. pneumoniae* ATCC 13883 (URE pos., IND neg.)	*E. coli* ATCC 25922 (URE neg., IND pos.)
Urease/TDA (Urea 4 mg, L-Tryptophane 1 µg)	URE/ TDA	Standard Read URE first, then+III	4 h (or overnight)	Red, purple Red, brown	Yellow Yellow, orange	*Proteus vulgaris* ATCC 13315 (URE pos., TDA pos.) *K. pneumoniae* ATCC 13883 (URE pos., TDA neg.)	*E. coli* ATCC 25922 (URE neg., TDA neg.)
Voges-Proskauer (Sodium Pyruvate 2 mg, Creatine 0.1mg)	VP	Standard+VI	4 h	Red, pink	Colourless (no change in colour)	*Enterobacter cloacae* ATCC 13047	*E. coli* ATCC 25922

Procedure:

Standard: Prepare a bacterial suspension (at least McFarland 4) from an overnight plate in 0.25 ml saline in a tube. Add one Diatabs, close the tube and incubate at 35-37 °C.

Standard+oil: Prepare a bacterial suspension (at least McFarland 4) from an overnight plate in 0.25 ml saline in a tube. Add one Diatabs and 3 drops of paraffin oil, close the tube and incubate at 35-37 °C.

a) Prepare the bacterial suspension in distilled water. After incubation add one drop of ferrous ammonium sulphate solution 5% w/v in purified water (freshly prepared and stored at –20 °C). For Corynebacteriae use inoculum McFarland 8.

b) Overnight only strong red or purple are positive.

Reagents to be added after incubation:

I. Add 3 drops Aminopeptidase reagent (Rosco #92231) and read colour reaction within 5 min.

IIa. Add 3 drops of Kovacs' reagent (Rosco #92031), shake and read colour of the surface layer after 3 min.

IIb. Add 4 drops of Kovacs' reagent (Rosco #92031), shake and wait for up to 10 min.

III. Add 2 drops of Ferric Chloride 10% solution and read colour within 5 min.

IV. Add 5 drops of Ninhydrin 3.5% solution (Rosco #91731), close the tube and reincubate for 10 min. Read within 5 min.

V. Add 1 drop of N,N-Dimethyl-α-Naphthylamine and 1 drop Sulfanilic solution. Read within 2 min.

VI. Add 2 drops of alpha-naphthol solution (5% in ethanol) and afterwards 1 drop of 40% KOH and shake.