Table 10. Gram-positive bacilli-a guide to identification testing and selected notes 1-10 (revised October 2008).
(R=resistant; S = susceptible; n/a not available; +, positive; -, negative)

Organism or group/ Selected tests	Catalase	Bile esculin/ Esculin hydrolysis	Motility (broth)	Penicillin	Vancomycin	Other
Aerobic actinomycetes	*Rhodococcus*+					**See Note 1.** (including *Nocardia, Rhodococcus*, and *Streptomyces*)
Aerotolerant Clostridia e.g., *C. tertium*	-	n/a	n/a	*C. tertium* R	n/a	Compare aerobic and anaerobic growth; better growth anaerobically *C. tertium* metronidazole, clindamycin R
Arcanobacterium haemolyticum	-	-	-	See other	n/a	Beta-hemolysis best in CO_2 CAMP inhibition reaction Low MICs for beta-lactams; however, frequent treatment failures
Bacillus anthracis ***See SAFETY***	+	n/a	-	S	S	No hemolysis – or very weak Aerotolerance tests; larger aerobically Lecithinase +
Bacillus cereus	+	n/a	+ 35°C	R	S	Hemolysis +; Lecithinase +
Bacillus thuringiensis	+	n/a	+	n/a	S	Parasporal crystals in sporulated cultures
***Corynebacterium* - 67 species**	+	Esculin hydrolysis V	- (medically relevant species)	n/a see other	n/a	*Corynebacterium jeikeium* R penicillin (*Microbacterium resistens* intrinsically R to vancomycin)
Erysipelothrix rhusiopathiae	-	-	**-"pipe cleaner"** pattern in gelatin stab at 22°C	S	R	**H_2S + in TSI** No growth at 4°C (range 5-42°C) Growth in NaCL up to 8.5% Variable Gram morphology and staining
Lactobacilli	-	V	n/a	n/a	R/s	Grow under anaerobic conditions Most are intrinsically R to vancomycin (>256 µg/mL).
Listeria monocytogenes	+ (neg. variant recently reported)	+	+ **tumbling in wet mount Motile at 28°C, less so at 37°C**	S (bacteriostatic)	S	Growth at 4°C Subdued beta-hemolysis that may resemble group B streptococci CAMP test with *S. aureus* + Glucose +, VP+, MR + Rhamnose + , Oxidase, indole, H_2S all negative Cephalosporins are ineffective in vivo
Rothia dentocariosa	V	Esculin hydrolysis +	-	n/a	S	
Rapidly growing Mycobacteria (*M. chelonae, M. abscessus* group and *M. fortuitum* group) 22 pathogenic species (known)						**See Note 2.**

1. **Aerobic actinomycetes** Tests include: modified (1% H_2SO_4) acid-fast test (may be weak) positive for *Nocardia* (use as a positive control), *Rhodococcus, Gordonia*, and *Tsukamurella*; *Streptomyces* (use as a negative control) is negative; also useful are pigment production and aerial hyphae (*Nocardia* and *Streptomyces*). Molecular techniques are currently (2008) the only methods that can provide definitive identification of most isolates of aerobic actinomycetes. Modified acid-fast stain performed on direct samples may be more accurate than performing the test on colonial growth*. Especially look for macrophages and mononuclear cells containing beaded cells with strongly acid-fast granules within non-acid fast or weakly acid-fast rods. In cases of suspected actinomycetoma look for granules in the aspirated material. Prepare smears from crushed granules.
2. **Rapidly growing Mycobacteria** * (*M. chelonae, M. abscessus* group and *M. fortuitum* group) RGM grow readily on blood or chocolate agar in 3 to 5 days and <7-days on L-J medium when sub-cultured and placed in the optimal incubation temperature. *M. chelonae* is the slowest growing member of this group and generally requires a lower temperature (i.e., 28 to 30°C) for primary isolation. All members of the *M. fortuitum* group and the *M. chelonae-M. abscessus* group are strongly positive for arylsulfatase production at 3 days [1]. *M. chelonae-M. abscessus* group show no zone of inhibition to polymyxin B disk (diffusion method) while the *M. fortuitum* group usually show a zone of $\geq$ 10 mm around the disk [1]. *M. chelonae* can be separated from *M. abscessus* by phenotypic tests: citrate utilization and growth in 5% NaCl. *M. chelonae* is citrate positive and does not grow in 5% NaCl; *M. abscessus* is citrate negative and will grow in the presence of 5% NaCl. RGMs may appear as beaded, occasionally filamentous, gram-positive rods on Gram stain and therefore be confused with a nonmycobacterial aerobic gram-positive rod. Gram stain may not detect the presence of *Mycobacterium* sp. because of the predominance of mycolic acid in the cell wall inhibits the uptake of the stains. Individual bacilli may display heavily stained areas alternating with areas of understaining, giving a beaded appearance, although RGM may occasionally lose the property of staining acid fast. There are also several nonmycobacterial organisms with various degrees of acid fastness, primarily *Rhodococcus* sp., *Nocardia* sp., and *Legionella micdadei*.
3. **Gram stain** is considered to be one of the essential procedures for the identification of this group of organisms; however, it is most reliable and consistent from growth in a broth medium where characteristics are enhanced.
4. **Morphology** A Gram stain of growth around a **10 µg penicillin disk** may also be helpful to determine cellular morphology.
5. ***Erysipelothrix*** cells stain gram-positive but can decolorize and appear gram-negative, with gram-positive granules giving a beaded effect. Smooth colonies may smear as rods or coccobacilli, sometimes in short chains while rough colonies may appear as long filaments (60µm).
6. **Aerobic spore-forming** bacilli observe hemolysis and set up motility test using motility medium. If motile, report as ***Bacillus*** sp. If non-motile, nonhemolytic, and large bacilli send to reference laboratory for identification.
7. **Aerotolerance tests** should be performed to rule out anaerobic gram-positive bacilli. Aerotolerant clostridia (e.g., ***C. tertium***) produce much better growth and sporulate only under anaerobic conditions, whereas *Bacillus* sp. generally have larger colonies and produce spores when incubated aerobically.
8. *It may be necessary to forward isolates to a reference laboratory for identification.
9. **SAFETY** Laboratorians are reminded to adhere to good safe handling practices when *Bacillus* species is isolated. Use the biological safety cabinet for any manipulation that may create aerosols. Visit the Public Health Agency of Canada website for copies of the *Laboratory Biosafety Guidelines*, 3rd edition (2004) and information on Bioterrorism and Emergency Preparedness.
10. **This table is presented only as a guideline to identification. Recent publications and identification schemas should also be consulted.**