WELLS	DESCRIPTION	RESULTS	INSTRUCTIONS SUGGESTIONS	POSSIBLE RESULTS AND ADDITIONAL INSTRUCTIONS (*)
1	Selective medium for the growth of *Streptococcus spp* and *Staphylococcus spp*. The medium components inhibit most gram negative bacteria (check with the result of the well 9)	POSITIVE: turbidity of the medium and/or micro-colonies in the bottom of the well. NEGATIVE: No turbidity is observed.	Perform Gram stain. Perform Catalase Test. Perform Free Coagulase Test. Perform agglutination latex test	Observation of bacterial structures. Test results correlate with the microscopic observation. Catalase test and Coagulase test can be performed using the culture medium of the well. (Samples with Gram-negative strains present in high concentrations may cause turbidity in the medium) Observation of the well to observe any micro colonies formations in the background is recommended. The test tube coagulase helps to rule out the possible presence of positive strains to slide coagulase test, or positive latex reagents and do not belong to the species *Staphylococcus aureus.*
2/3	Selective media for growth of *Streptococcus pneumoniae.* Presumptive identification is made observing the results of both wells and results in well 1. PRESUMPTIVE IDENTIFICATION OF *S.PNEUMONIAE* IS EVIDENCED BY THE RESULT OF BOTH WELLS. RED COLOUR OR TURBIDITY IN THE WELL 2 IS A NEGATIVE RESULT.	**WELL 2:** **POSITIVE: Light yellow color transparent** **NEGATIVE: Red color or turbidity in the culture medium**	Light Yellow color transparent of the well 2 is presumptively *Streptococcus pneumoniae*	Correlate the obtained results in well 2 with performed tests from the culture of well 3. **The development of a red color or turbidity in the culture medium of well 2 corresponds to the growth of microorganisms not belonging to the species *S.pneumoniae*** Latex reagents for *S.pneumoniae* do not detect all existing serotypes, especially when are strains of
		WELL 3: **POSITIVE: Light yellow color and**	Gently aspirate the well content: Perform Gram Staining.	

		Turbidity. **NEGATIVE: Light yellow color.** NO TURBIDITY	Perform staining for bacterial capsules observation. Perform confirmatory test for the detection of antigens by latex agglutination. Perform capsular swelling test.	nasopharyngeal carriers. Capsular swelling test depends on the availability of antisera (OMNI, sera of groups and/or types). Some demanding strains of *S. pneumoniae* do not grow into well 1. All confirmatory tests for this agent must be made from well 3. Observation of the well is recommended to observe any eventual micro colonies formations on the background.
4	Selective medium for PYR TEST.	**POSITIVE: Red Color** **NEGATIVE: Yellow Color**	Presumptive identification of *Streptococcus pyogenes* is defined with the results obtained in wells 4 and 5, in addition to the results of the well 1 (gram-positive cocci in chains, catalase negative). Perform a confirmatory test with latex agglutination or immunochromatographic cards from the culture medium of well 5.	Other PYR positive strains present in the sample may be positive in the well 4. Other PYR positive strains such as *Staphylococcus lugdunensis*, *Enterococcus spp.*, *Citrobacter spp.* and *Enterobacter spp.* can give positive reactions. Observation of the well to identify any micro colonies formations in the background is recommended. Some strains do not belong to the genus *Streptococcus spp* and can cause turbidity in the well 5.
5	Selective medium for the growth of *Streptococcus spp.*	**POSITIVE: Turbidity** **NEGATIVE: Yellow Transparent color**		
6	Selective medium for presumptive identification of *Streptococcus agalactiae*	**POSITIVE: Green** **NEGATIVE: The appearance of other colorations, or turbidity in the culture medium**	Perform sodium hippurate test on disk from the contents of well 1. Perform confirmatory test with latex agglutination or immunochromatographic cards from well 1.	The presence of a green coloration in the well 6 is a presumptive diagnosis of *S. agalactiae*. It is recommended a correlation with the results of the well 1 as well as performs the Sodium Hippurate test, the latex agglutination and chromatographic card from the well 1. Some not hemolytic strains of *Streptococcus agalactiae* do not produce coloration in the well 6. In the case that the presence of *S. agalactiae* is suspected and no color change is observed in the well 6, the sodium hippurate test and latex agglutination test should be performed from the well 1.

7	Selective medium for growth *of Haemophilus influenzae*. Presumptive identification is made observing positivity of well 7, negativity of well 8, and positivity of well 9.	**POSITIVE: Light Intense Red** **NEGATIVE: Yellow or orange color.**	Perform confirmatory test with latex agglutination and Gram staining carefully aspirating the content of the wells 7.	The average content in the well 7 contains X and V factors and other growth factors for all strains of *Haemophilus spp*. All species of the genus *Haemophilus* (capsulated or not capsulated) cause coloration to red. Non capsulated strains are negative to latex reagents available, however can be a frequent cause of LRTI. The Gram staining helps show *pleomorphic coccobacilli*. *H.influenzae* strains grow perfectly in well 7 causing the appearance of a red color and in well 9 a yellow color is observed, while they are unable to grow in the well 8. The presumptive identification of *H. influenzae* should be made by observing the wells 7, 8 and 9, and Gram staining and latex test. Some Gram-positive strains resistant to selective agents of well 7 can cause a change of the medium chromogenic, causing a red color appearance, growing in well 8 but produce no color change in the well 9.
8	Selective medium for growth of *Haemophilus spp.*	**POSITIVE: Light Transparent Yellow or/and Light Turbid Yellow** **NEGATIVE: Pink/red color**	Perform confirmatory latex agglutination test and Gram staining carefully aspirating the contents of the well.	*Haemophilus spp* strains that do not require factor X (*H.parainfluenzae*) are capable of growing in the well 8 and 9 while *H.influenzae* only grows in the 7 and 9.
9	Well 9: Selective Medium for the presumptive identification of gram-negative	**POSITIVE: INTENSE YELLOW**	Perform confirmatory latex agglutination	Selective medium containing growth factors X and V

	bacteria.	**NEGATIVE: TRANSPARENT OR TURBID WHITE**	test and Gram staining carefully aspirating the contents of the well.	and enrichment supplements necessary for gram-negative bacteria of difficult growth, which can be found relatively often in the respiratory tract. Other gram-negative strains present in the sample can cause a color change in the well.
10/11	Selective medium containing chromogenic substrate for presumptive identification of *Staphylococcus aureus.*	**WELL 10** **POSITIVE: Violet** **NEGATIVE: no color****	The presumptive identification of *Staphylococcus aureus* is evidenced by the positive results of the wells 10 and 11, the results of well 1, and negativity of well 4. It is recommended to perform a coagulase test or latex agglutination test from the medium contained in the well 1 or 9.	Positive reactions may occur in well 10 when *Enterococcus spp* strains are present in the sample in concentrations higher of 10^3 CFU. However, in these cases the well 11 is negative, while the well 4 is positive and the well 1 tests indicate the presence of *Streptococcus spp*. **Colors different from violet must be intended as negative results
	Selective medium containing growth indicators for the presumptive identification of *Staphylococcus aureus.*	**WELL 11** **POSITIVE: Black, precipitation can be seen in the bottom of the well.** **NEGATIVE: White, light yellow or very light gray**		
12/15	Selective medium containing Arginine for the presumptive identification of *Mycoplasma hominis.*	**WELL 12** **POSITIVE:** RED **NEGATIVE**: YELLOW	Positive. Presumptive diagnosis of *Mycoplasma hominis*.	The presumptive diagnosis should be made based on the results of the wells 12-15. Is recommended the use of complementary identification methods especially for *Mycoplasma spp.* available in the laboratory. Culture of plates is recommended to observe the colonies characteristics. Culture of plates is recommended to observe the characteristics of colonies. Is recommended the microscopic observation of the wells to identify any micro colonies formations at the bottom of the wells 13, 14 and 15. *M.hominis* strains are presumptively identified by well 12 positivity and negativity of the wells 13, 14 and 15. *Mycoplasma spp.* species such as *M. pneumoniae* should result negative in well 12 and positive in
	Selective medium containing glucose for presumptive identification of *Mycoplasma spp*.	**WELL 13** **POSITIVE**: YELLOW **NEGATIVE**: RED	Positive. Presumptive diagnosis for *Mycoplasma spp*.	
	Selective medium containing glucose and growth factors for presumptive identification of *Mycoplasma spp*.	**WELL 14** **POSITIVE**: YELLOW **NEGATIVE**: RED	Positive. Presumptive diagnosis for *Mycoplasma spp*.	
	Selective medium containing growth factors and chromogens, for presumptive identification of *Mycoplasma spp*.	**WELL 15** **POSITIVE:** YELLOW **NEGATIVE:** RED	Positive. Presumptive diagnosis for *Mycoplasma spp*.	

				wells 13, 14 and 15 after 72 hours of incubation. Other species of *Mycoplasma spp* result negative in well 12, positive in well well 13, variable in well 14 and negativity in the well 15. The presence in the sample of resistant strains and multi-resistant strains as *Pseudomonas spp, Acinetobacter spp, Staphylococcus MRSA*, can cause a colorimetric reaction in the wells of *Mycoplasma spp*. sector, that is evidenced by the change of color to strong yellow of *Mycoplasma spp.* sector after 24 hours of incubation. The positivity of wells 13,14 y 15 after 24 hours is indicative for species of other strains not belonging to the group of mycoplasmas. *M.hominis*: Grows from 24/48 hours. *M.pneumoniae*: Grows from 72 hours. Positive in the sector of *Mycoplasma spp.* should be confirmed by additional identification methods
16	Selective medium for presumptive identification of *Pseudomonas spp*.	Aquamarine green evidently TURBID.	Perform oxidase test	Some bacteria such as *Enterococcus spp* and some enterobacteria can cause a light green color in the well but is weak and doesn´t cause turbidity. Correlate the result of the well 9.

ANTIMICROBIAL SUSCEPTIBILITY			
	Antimicrobial	RESISTANT	SUSCEPTIBLE
17	PTZ 128/4 µg/mL	Beet Turbid Red	White or transparent Pink
18	CF 32 µg/mL	Beet Turbid Red	White or transparent Pink
19	CRO 64 µg/mL	Beet Turbid Red	White or transparent Pink
20	VAN 2 µg/mL	Beet Turbid Red	White or transparent Pink

21	CD 0.5 µg/mL	Beet Turbid Red	White or transparent Pink	
22	AZM 32 µg/mL	Beet Turbid Red	White or transparent Pink	
23	E 8 µg/mL	Beet Turbid Red	White or transparent Pink	
24	GEN 16 µg/mL	Beet Turbid Red	White or transparent Pink	
25	OFX 8 µg/mL	Beet Turbid Red	White or transparent Pink	
26	SXT 4/76 µg/mL	Beet Turbid Red	White or transparent Pink	
IDENTIFICATION OF CANDIDA spp.				
27	Culture medium containing chromogenic and selective agents for *Candida albicans*.	**POSITIVE: Green** **NEGATVO: White*****	Presumptive diagnosis *Candida albicans*	Perform microscopic observation (40X) of the well 28 to observe characteristic structures (chlamydospores and /or hyphae). ***Colors different from green must be intended as negative results
28	Culture medium containing selective and chromogenic agents for *Candida spp*.	**POSITIVE: Turbid** **NEGATIVE: Transparent Yellow**	Microscopic confirmation of typical structures of *Candida spp*.	The medium is designed for the growth of all species of *Candida spp*. Perform microscopic observation (40X)
ANTIFUNGAL SUSCEPTIBILITY TEST				
	Antifungal	RESISTANT	SUSCEPTIBLE	
29	FLC 64 µg/mL	Turbid Beet Red	White or transparent Pink	
30	AMB 2 µg/mL	Turbid Beet Red	White or transparent Pink	
31	KTC 1 µg/mL	Turbid Beet Red	White or transparent Pink	
32	ITC 1 µg/mL	Turbid Beet Red	White or transparent Pink	

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*** Whenever possible inoculation of samples in traditional culture media such as sheep blood agar at 5% is recommended.**

TABLE OF SYMBOLS

REF Code LOT LOTE Number Cont. Content Expiration Date

Conservation Temperature IVD For diagnostic *in vitro* use

Do not reuse Fragile, careful using Interior literature

Producer Enough content for <n> tests

CE

Conform to the Directive 98/79/EC on *in vitro* diagnostic medical device

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