Quanti-CultiControl

Technical Sheet 02

Quanti-CultiControl freeze-dried microorganisms
Packaging: 1 vial containing 1 pellet + 1 rehydration fluid vial
Quantitative: <100 CFU / 0.1 mL inoculum
Applications: Growth Promotion Testing, Microbial Limits Testing, Microbial Enumeration Testing

BioSafety Levels

The Liofilchem® CultiControl freeze-dried microorganisms have a BioSafety level (BSL) of 1 or 2.

BSL 1 organisms have no, or low, risk to individuals and communities. BSL 1 organisms may cause disease in individuals with immune systems that are suppressed or compromised.

BSL 2 organisms pose a moderate risk of individual infection, but low risk of community infection.

Liofilchem adheres to the BSL level designation as determined by the Reference Culture Collection from which the microorganism strain was obtained. Responsibility for safe handling of biological agents ultimately rests with the user. All infectious materials should be handled under the supervision of a competent and knowledgeable microbiologist.

Recommended Growth Methods

Primary growth on a nonselective agar medium is preferred. Primary growth in a fluid medium should only occur in special instances or when recommended. Because of the manipulations required during hydration, it is difficult to obtain purity of a lyophilized strain in a fluid medium. A contaminant may completely overgrow and obscure the presence of the lyophilized strain.
A list of microorganisms and relevant Recommended Growth Method is showed at page 4.

Method 1
Tryptic Soy Agar (Soybean Casein Digest Agar), nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar - 35°C in aerobic atmosphere – 24 to 48 hours.

Method 2
Nonselective Sheep Blood Agar - 35°C in aerobic atmosphere – 24 to 72 hours. Growth of some species such as *Streptococcus* and *Arcanobacterium* are enhanced by CO_2 enrichment of the incubation atmosphere. 5% CO_2 is recommended for the culture of *Streptococcus pneumonia*e and other streptococcal species of the viridians group.

Method 3
Chocolate Agar - 35°C in 5% to 7% CO_2 – 24 to 48 hours.

Method 4
Anaerobic Blood Agar 35°C in Anaerobic Environment – 48 to 72 hours.
Some obligate anaerobes may require 5 to 7 days to demonstrate sufficient growth.
Fresh prepared Nutrient Agar, Tryptic Soy Agar (Soybean Casein Digest Agar), Standard Methods Agar (Plate Count Agar) are appropriate alternatives for some *Clostridium* species together with an additional period (24 hours) of incubation.

Method 5
Sabouraud Dextrose Emmons Agar - 25°C in aerobic atmosphere – 2 to 7 days.
Nonselective Sheep Blood Agar is an appropriate alternative.
Nutrient Agar, Tryptic Soy Agar, Potato Dextrose Agar and Standard Plate Count Agar are appropriate alternatives together with an additional period (24 hours) of incubation.
Sabouraud Dextrose Emmons Agar is the best medium for growth of *Saccharomyces* sp.

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Method 6
Chocolate Agar - 35°C in Microaerophilic Environment – 48 to 72 hours.

Method 7
Lowenstein Jensen Agar or Middlebrook Agar - 35°C in 5 to 7% CO_2 or aerobic atmosphere – up to one week. *M. fortuitum* subsp. *fortuitum, M. peregrinum and M. smegmatis* will also grow on Tryptic Soy Agar (Soybean Casein Digest Agar) as well as Lowenstein Jensen and Middlebrook Agar but additional incubation time may be required.

Method 8
Buffered Charcoal Yeast Extract Agar - 35°C in aerobic atmosphere – 3 to 5 days.

Method 9
V Agar or Chocolate Agar - 35°C in 5% to 7% CO_2– 48 hours.

Method 10
Rehydrate in sterile Brain Heart Infusion Broth, Tryptic Soy Broth (Soybean Casein Digest Agar) or 0.85% Saline. Rehydration with water may result in decreased or no recovery. Grow on Tryptic Soy Agar (Soybean Casein Digest Agar) - 35°C in aerobic atmosphere – 24 to 48 hrs. *Vibrio* sp. also grows on Marine Agar.

Method 11
The primary growth medium is MRS (Man, Rogosa, Sharpe) Broth. Incubate at 35°C in aerobic atmosphere for 48 hours. Transfer to either Columbia CNA with Sheep Blood or Tryptic Soy Agar with Sheep Blood. Incubate at 35°C in 5 to 7% CO_2 for 48 hrs. A few *Lactobacilli* species, such as *L. fermentum*, *L. paracasei* subsp. *paracasei*, *L. plantarum*, *L. rhamnosus*, and *L. sakei*, do not need to be started in Lactobacilli MRS broth. They may be plated directly to Columbia CNA with Sheep Blood or Tryptic Soy Agar with Sheep Blood and incubated at 35°C in 5 to 7% CO_2 for 48 hrs.

Method 12
Potato Dextrose Agar - 55 C in aerobic atmosphere – 24 to 48 hours.

Method 13
Rehydrate I pellet of *M. hominis* or *Ureaplasma* sp. in 10B Arginine Broth. Make serial dilutions (for example, 1:10, 1:100, 1:1000, 1:10,000). Incubate at 35 C in aerobic atmosphere. As soon as the Arginine vial turns pink (24 to 48 hours), sub 0.1 mL of broth to A8 Agar and streak for isolation. Do not use cotton swab or wooden shaft. Incubate mycoplasma at 35 C in 5 to 7% CO_2. Incubate ureaplasma at 35 C anaerobically for up to 96 hours. In order to see colonies, examine plates microscopically.

Method 14
Rehydrate 1 pellet of *M. pneumoniae* in SP4 Glucose Broth. Make serial dilutions (for example, 1:10, 1:100, 1:1000, 1:10,000). Incubate at 35°C in aerobic atmosphere. As soon as the broth turns from red to yellow (1-4 weeks), sub 0.2 mL of broth to SP4 Glucose Agar and streak for isolation. Do not use cotton swab or wooden shaft. Incubate at 35°C in CO_2 atmosphere, preferably in a candle jar, for 5 to 15 days. In order to see colonies, examine plates microscopically.

Method 15
Rehydrate 1 pellet of *M. orale* in 10B Arginine Broth. Make serial dilutions (for example, 1:10, 1:100, 1:1000). Incubate at 35°C, in aerobic atmosphere. As soon as the broth turns from yellow to pink (48 to 72 hours), sub 0.2 mL of broth to SP4 Glucose Agar and streak for isolation. Do not use cotton swab or wooden shaft. Incubate plates at 35°C in anaerobic conditions for 3 to 6 days. In order to see colonies, examine plates microscopically.

Method 16
Leeming Notman Agar - 30°C in aerobic atmosphere – 72 hours.

Method 17
Rehydrate 1 pellet of *M. gallisepticum* in SP4 Glucose Broth. Make serial dilutions (for example, 1:2, 1:4). Incubate at 35°C in aerobic atmosphere. As soon as the broth turns from red to yellow (4 days to 2 weeks), sub 0.2 mL of broth to SP4 Glucose Agar and streak for isolation. Do not use cotton swab or wooden shaft. Incubate at 35°C in CO_2 atmosphere, preferably in a candle jar, for 3 days to 2 weeks. In order to see colonies, examine plates microscopically.

Method 18
Rehydrate 1 pellet of *M. hyorhinis* in SP4 Glucose Broth. Make serial dilutions (for example, 1:10, 1:100, 1:1000). Incubate at 35°C in aerobic atmosphere. As soon as the broth turns from red to yellow (4 days to 2 weeks), sub 0.2 mL of broth to SP4 Glucose Agar and streak for isolation. Do not use cotton swab or wooden shaft. Incubate at 35°C in CO_2 atmosphere, preferably in a candle jar, for 2 to 10 days. In order to see colonies, examine plates microscopically.

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Method 19
Rehydrate 1 pellet of *M. synoviae* in SP4 Glucose Broth. Make serial dilutions (for example, 1:2, 1:4, 1:8, 1:16, 1:32). Incubate at 35°C in 5 to 10% CO_2 for 7 days. After 7 days (no color change will be noted), sub 0.2 mL of broth to SP4 Glucose Agar and streak for isolation. Do not use cotton swab or wooden shaft. Incubate at 35°C in CO_2 atmosphere, preferably in a candle jar, for 1 to 4 weeks. In order to see colonies, examine plates microscopically.

Method 20
Chocolate agar, Sheep Blood Agar, Tryptic Soy Agar, Bordet Gengou Agar with 15% Defibrinated Sheep Blood - 35°C in aerobic atmosphere – 24 to 48 hours. Standard Methods (Plate Count Agar) or Nutrient Agar are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 21
Chocolate or Bordet Gengou Agar with 15% Defibrinated Sheep Blood - 35°C in aerobic atmosphere – 2 days to one week. *B. pertussis*, and *B. pertussis*, require Bordet Gengou Agar with 15% Defibrinated Sheep Blood.

Method 22
Prepare ISF (modified Infant Soy Formula) Broth using the following steps: 1) fill tubes with 10 mL Infant Soy Formula, 2) place a four-penny nail in each tube, and 3) sterilize the broth. Infant Soy Formula may be purchased at a grocery store. A four-penny nail is approximately 1.5 inches or 38 mm in length. It should contain steel or iron.
Inoculate ISF Broth with one pellet. Make two dilutions, 1:10 and 1:100. Plate undiluted sample and plate the 1:10 and 1:100 dilutions. It is necessary to plate the diluted samples because at higher concentrations the colonies are pin-point which makes colony characteristics difficult to see. Grow at 55°C in anaerobic conditions for 48 hours. The broth will turn grey, indicating growth. Sub with a swab to Sulfite Agar. Sulfite Agar is used for detecting thermophilic anaerobes which produce sulfite. Incubate the agar in anaerobic environment at 55°C for 7 days.

Method 23
Inoculate Mycoplasma Broth with a pellet. Prepare serial dilutions of 1:10, 1:100, and 1:1000 using the broth. Incubate at 35°C for 48 hours. Then plate 0.2 mL of the turbid broth culture to Mycoplasma Agar. Incubate agar in 5 to 7% CO_2 at 35° for 3 to 7 days. Do not use cotton swabs or wooden sticks. In order to see colonies, examine plates microscopically.

Method 24
Sheep Blood Agar supplemented with Pyridoxal - 35° C in 5% to 7% CO_2 – 24 to 48 hours.

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Quanti-CultiControl
freeze-dried microorganisms

Description	Quanti-CultiControl™ Ref.	notes	BioSafety Level	recommended growth method
Aspergillus brasiliensis derived from ATCC® 16404™*	89501		1	5
Bacillus cereus derived from ATCC® 10876™*	89502		1	1
Bacillus subtilis subsp. spizizenii derived from ATCC® 6633™*	89503		1	1
Bacteroides fragilis derived from ATCC® 25285™*	89505		2	4
Bifidobacterium animalis subsp. animalis derived from ATCC® 25527™*	89539		1	4
Brevundimonas diminuta derived from ATCC® 19146™*	89506		1	1
Burkholderia cepacia derived from ATCC® 25416™*	89507		2	1
Candida albicans derived from ATCC® 10231™*	89508		1	5
Candida albicans derived from ATCC® 2091™*	89510		1	5
Clostridium perfringens derived from ATCC® 13124™*	89512		2	4
Clostridium sporogenes derived from ATCC® 11437™*	89513		1	4
Clostridium sporogenes derived from ATCC® 19404™*	89514		1	4
Enterobacter aerogenes derived from ATCC® 13048™*	89516		1	1
Enterococcus faecalis derived from ATCC® 29212™*	89517		2	1
Enterococcus faecalis derived from ATCC® 7080™*	89518		2	1
Escherichia coli derived from ATCC® 8739™*	89519		1	1
Geobacillus stearothermophilus derived from ATCC® 12980™*	89521		1	1
Geobacillus stearothermophilus derived from ATCC® 7953™*	89522		1	1
Kocuria rhizophila derived from ATCC® 9341™*	89523		1	1
Lactobacillus fermentum derived from ATCC® 9338™*	89524		1	11
Listeria monocytogenes derived from ATCC® 19115™*	89525	serotype 4b	2	1
Micrococcus luteus derived from ATCC® 4698™*	89526		1	1
Pseudomonas aeruginosa derived from ATCC® 27853™*	89527		2	1
Pseudomonas aeruginosa derived from ATCC® 9027™*	89528		2	1
Salmonella enterica subsp. enterica serovar Choleraesuis derived from ATCC® 10708™*	89529	H_2S negative	2	1
Salmonella enterica subsp. enterica serovar Typhimurium derived from ATCC® 14028™*	89531		2	1
Salmonella enterica subsp. enterica serovar Typhimurium derived from ATCC® 13311™*	89530		2	1
Staphylococcus aureus subsp. aureus derived from ATCC® 25923™*	89533	recommended for CAMP test	2	1
Staphylococcus aureus derived from ATCC® 6538™*	89535		2	1
Staphylococcus aureus derived from ATCC® 6538P™*	89534		2	1
Staphylococcus epidermidis derived from ATCC® 12228™*	89537		1	1
Streptococcus pyogenes derived from ATCC® 19615™*	89538	group A	2	2

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