

Chapter 26

Culture

SUITABLE MEDIA FOR SPECIFIC ORGANISMS

Acanthamoeba: 1.5% agar with *Escherichia coli*

Actinobacillus: blood agar CO₂ + moisture

Actinomyces: blood agar + vitamin K and blood agar + colistin and nalidixic acid anaerobic + CO₂ (enriched thioglycolate broth suitable only for pure cultures)

Adenovirus: oropharyngeal, nasopharyngeal, throat gargle, throat swab, faeces, conjunctival swab, sputum in HEP2, embryonic kidney cells

Aeromonas: blood agar + 10 mg/L ampicillin, MacConkey agar, SS agar

Alcaligenes: MacConkey agar

Anaerobes: CDC anaerobic blood agar, phenylethyl agar, vancomycin kanamycin agar (Gram negative), paromomycin vancomycin blood agar (Gram negative), enriched thioglycolate broth. Selective media should always be used for isolation, since only 61% of Gram negative and 73% of all anaerobes will be isolated on non-selective media. Using an anaerobic jar, within 30 minutes it is possible to check the system is working by reduction of methylene blue indicator from blue to white, condensation on surface of jar and jar becoming warm. Palladium catalysts are readily inactivated by excessive moisture and H₂S.

Anaerobic Streptococci: sheep blood agar with neomycin

Arbovirus: blood, brain post mortem in chick embryo chorioalantoic membrane or yolk sac, cell culture

Avian Bronchitis-like Virus: throat swab or washings in diploid human embryonic fibroblasts, organ culture of trachea or nasal epithelium

Bacillus: blood agar aerobic

Bacteroides: sheep blood agar with kanamycin and vancomycin anaerobic

Bordetella: blood agar (some species), Bordet-Gengou potato-glycerol blood agar, charcoal agar with antibiotics in moist atmosphere

Brucella: blood agar CO₂

Calymmatobacterium granulomatis: egg yolk-containing media aerobic

Campylobacter: enrichment in medium of Martin et al, Skirrow's medium microaerophilically at 42°C

Capnocytophaga: blood agar CO₂

Cardiobacterium hominis: blood agar CO₂ + moisture

Chlamydia: McCoy's tissue culture with Stoxil

Chromobacterium violaceum: MacConkey agar, SS agar

Clostridium: blood agar anaerobic, cooked meat medium

Clostridium difficile: faeces on blood agar + cycloserine + cefoxitin + fructose anaerobic

Clostridium perfringens (foodborne illness): faeces and food on tryptose sulphite cycloserine agar anaerobic

Corynebacterium: blood agar, cysteine tellurite blood agar, Loeffler's serum, Tinsdale aerobic

Coxsackievirus: faeces, throat swab, CSF, heart post mortem in monkey or human cell culture

Cytomegalovirus: saliva, throat swab, urine, leucocytes, liver, etc in human embryonic fibroblasts

Diphtheria: throat membrane fragments on Tinsdale, Loeffler's

Echovirus: faeces, throat swab, CSF in monkey or human cell culture

Eikenella corrodens: blood agar CO₂

Entamoeba: Balamuth's, egg yolk medium

Enterobacteriaceae: MacConkey, eosin methylene blue

Enterohaemorrhagic Escherichia coli: 0.5% sorbitol MacConkey agar

Escherichia: MacConkey or eosin methylene blue aerobic

Erysipelothrix rhusiopathiae: blood/glucose agar CO₂

Francisella tularensis: nodules, pustules, ulcers, lymph node aspirate, blood, pleural aspirate, sputum on glucose-cystine-thiamine blood agar, cystine-heart blood agar, cystine-yeast agar + ? -ketoglutarate, chocolate agar + Isovitalex, Thayer-Martin medium aerobic

Fungi: Sabourand, Mycosel, brain heart infusion, malt extract agar; requirements include detailed clinical notes, adequate amount of suitable clinical material, thorough direct microscopic examination of potassium hydroxide-ink mounts or stained smears, histopathology of biopsy tissue stained with special fungal stains, and use of antibacterials in primary isolation media

Fusobacterium: blood agar anaerobic

Gardnerella vaginalis: heart infusion agar + 6% rabbit or human blood CO₂

Haemophilus: enriched chocolate agar CO₂

Haemophilus ducreyi: enriched chocolate agar + 1% bovine haemoglobin + 5% serum, Muller-Hinton agar + 5% chocolatised horse blood in high humidity at 33-35°C

Helicobacter pylori: multiple gastric mucosal biopsies on chocolate agar or brain heart infusion agar ± nalidixic acid (50 mg/L), vancomycin (3 mg/L) and trimethoprim (5 mg/L) aerobic + CO₂ + moisture

Herpes simplex: vesicle fluid, throat swab, CSF, corneal scraping, brain post mortem in any cell culture (Cellmatics mink lung cells, MRC-5 most useful; shell vial centrifugation enhancement fastest and most sensitive procedure), chick embryo chorioallantoic membrane or yolk sac

Human papillomavirus: organ culture of infected skin treated with TPA to increase keratinocyte differentiation

Influenza: oropharyngeal, nasopharyngeal, throat swab or gargle specimens, lung tissue post mortem in chick embryo amnion, human, monkey, pig or calf kidney

Klebsiella: MacConkey or eosin methylene blue

Lactobacillus: special medium anaerobic + CO₂

Legionella: ACES-buffered charcoal yeast extract medium + ? -ketoglutarate (BCYE?) and BCYE? + cephamandole + polymyxin B + anisamycin (BMPA?) or BCYE? + glycine + vancomycin + polymyxin B + anisamycin (MWY)

Leishmania: Novy McNeil Nicolle medium, hamster inoculation

Listeria: previous storage of specimens at 4°C, enrichment in broth based on trypticase ± peptones + acriflavine dyes + nalidixic acid ± potassium thiocyanate ± cycloheximide, blood agar, blood agar + cycloheximide + colistin + cefotetan + fosfomicin + acriflavine aerobic

Marburg Virus: blood, serum, suspensions of heart, kidney, liver, spleen in Vero cells

Micrococcus: blood agar aerobic

Morbillivirus: throat swab, blood, brain or lung post mortem in human kidney or amnion cells

Mumps: saliva, CSF, urine in monkey or human kidney, chick embryo amnion

Mycobacterium: Lowenstein-Jensen, Gruft, Middlebrook 7H9, 7H10, 7H11, selective 7H11, Dubos, Wallenstein aerobic, isolator lysis centrifugation or other concentrate to Bactec 7H12, 13A

Mycoplasma: modified SP-4 broth and A7B agar

Neisseria: blood agar, chocolate agar, Thayer-Martin, Transgrow, New York City CO₂

Nocardia: blood agar, chocolate agar, mycobacterial media, modified Thayer-Martin medium, paraffin-containing medium, BMPA?, MWY charcoal yeast extract agar aerobic

Orf: vesicle fluid or pus in chick embryo chorioallantoic membrane

Papovaviridae: primary human foetal glial cells

Parainfluenza: oropharyngeal, nasopharyngeal, throat gargle, sputum in monkey or human kidney cells

Pasteurella: blood agar aerobic (except *P.aerogenes*— obligate anaerobe)

Pertussis: nasopharyngeal swab on charcoal agar + antibiotics

Plesiomonas shigelloides: MacConkey agar, SS agar

Pneumocystis jiroveci: Vero cell culture

Pneumovirus: throat swab, sputum in HEP2 cells

Poliomyelitis: throat swab, stool in monkey or human cell culture

Proteus: blood agar, MacConkey aerobic

Pseudomonas: blood agar, MacConkey aerobic
Reovirus: faeces, throat swab in monkey kidney cells
Rhinovirus: nose or throat swab in diploid human embryonic fibroblast culture at 33°C and pH7
Rickettsia: embryonated egg
Rotovirus: differentiating human colon carcinoma cell line + trypsin
Rubella: throat swab, blood, urine, any organ of rubella babies in RKI3, BHK21, green monkey kidney, rabbit cornea
Salmonella: enrichment in Gram negative broth or selenite broth, xylose lysine desoxycholate agar, MacConkey, SS agar aerobic
Shigella: enrichment in gram negative broth, xylose lysine desoxycholate agar, MacConkey, eosin methylene blue, SS agar aerobic
Simonsiella: sheep blood agar, BSTSY agar aerobic
Spirillum minus: animal inoculation
Staphylococcus: blood agar, mannitol salt agar aerobic
Streptobacillus moniliformis: blood agar aerobic + moisture
Streptococcus: blood agar anaerobic + CO₂
Streptomyces: blood agar aerobic
Trichomonas: Ruperberg broth
Trypanosoma: Novy McNeil Nicolle medium
Ureaplasma: modified SP-4 broth and A7B agar
Varicellavirus: vesicle fluid in human embryonic fibroblasts
Veillonella: selective media with lactic acid
Vibrio: alkaline media, thiosulphate citrate bile sucrose agar, sucrose teepol tellurite agar, most common media containing 0.5-1% salt
Yersinia: cold enrichment, blood agar aerobic, cefsulodin-irgasan-novobiocin medium

GENERAL NOTES FOR READING CULTURES

The first step is to read the request form, noting especially if the referring clinician has made any special requests, if the patient is on any antimicrobial therapy, any previous culture results, and the patient history (including immunocompromise and hospitalisation).

The result of the primary Gram stain should be consulted, noting the number and types of cells present and assessing whether the organisms seen in the Gram stain have grown on culture and vice versa. If Gram stain and culture do not correlate, the Gram stain should be checked and amended if necessary or a further effort made to isolate organisms seen which have not been cultured (eg., prolonged incubation, reinoculating specimen onto more appropriate media).

Culture plates should be read in conjunction with each other, noting, for example, whether organisms growing on blood agar also grow on MacConkey or colistin nalidixic acid agar, whether organisms growing on anaerobic plates are also growing on aerobic plates, whether haemolysis differs on blood agar and *Gardnerella vaginalis* agar.

All plates from cutaneous wounds that have moderate to numerous leucocytes seen in the Gram stain and no pathogen isolated should be reincubated for 5 days to check for *Mycobacterium* or *Nocardia*. If a patient has chronic ulcers and nothing has been isolated from previous swabs received, these plates should be reincubated also.

SUITABLE MEDIA FOR SPECIFIC SITES

Respiratory Specimens: Blood agar grows most significant aerobic respiratory flora except *Haemophilus influenzae*, which will grow but usually only as tiny colonies. It is important that the medium used shows the correct haemolysis. **Enriched chocolate agar + bacitracin** grows *Haemophilus influenzae* well, the bacitracin inhibiting Gram positive organisms, though some *Haemophilus influenzae* strains are also sensitive to it. **Colistin nalidixic acid agar** grows Gram positive, but not most Gram negative, organisms and can be useful with sputa containing enteric Gram negative bacilli and *Pseudomonas*. In patients where they are likely to be significant, enteric Gram negative bacilli and *Pseudomonas* can be isolated on **MacConkey agar**. Where appropriate, cultures for *Mycoplasma* can be set up on **A7B agar**.

Faeces: Xylose lysine deoxycholate medium relies on xylose fermentation, lysine decarboxylation and production of H₂S for primary differentiation of *Salmonella* and *Shigella* from non-pathogenic bacteria. Sodium

desoxycholate is included to inhibit conformers. **Salmonella shigella agar** contains bile salts to inhibit Gram positive organisms and coliforms and relies on lactose fermentation for primary differentiation. **CIN medium** contains an antibiotic supplement and sodium desoxycholate to select for *Yersinia enterocolitica*. **Skirrow's medium**, consisting of blood agar + vancomycin, polymyxin B and trimethoprim, is incubated at 42°C under microaerophilic conditions for the selective isolation of *Campylobacter*. Liquid specimens, or specimens submitted with a history of food poisoning after ingestion of seafood, should be screened for *Vibrio* using **thiosulphate citrate bile sucrose agar**, on which they produce colonies > 2 mm after 24 h incubation. **Clostridium difficile agar** consists of blood agar + D-cycloserine and cefoxitin, which inhibit almost all other organisms. The organism should be screened for if there is a history of diarrhoea following use of antimicrobials. Plates are incubated at 37°C anaerobically for 48 h. If *Aeromonas* is suspected, it may be cultured on **blood agar + ampicillin**.

Urine: Cystine lactose electrolyte deficient medium supports the growth of all urinary pathogens (with rare exceptions), giving good colonial differentiation and clear diagnostic characteristics. The presence of important contaminants, such as diphtheroids, *Lactobacillus* and *Micrococcus*, is also clearly elicited, giving an indication of the degree of contamination. It provides a non-inhibitory diagnostic agar for plate culture of urinary organisms. It is electrolyte deficient to prevent the swarming of *Proteus*. Suprapubic aspirates, ureteric specimens and other urines for which more extensive treatment is warranted may also be cultured onto enriched chocolate agar, anaerobic media and into thioglycolate broth.

Genital Specimens: Blood agar will grow most aerobes found in genital specimens, exceptions being *Neisseria gonorrhoeae*, which grows poorly after 48 h (*Neisseria meningitidis* grows well after 24 h), and *Haemophilus influenzae*, which grows poorly unless *Staphylococcus* is present, in which case satellitism may be observed (note that other organisms also produce satellitism). **Enriched chocolate agar + bacitracin** should be set up on females less than 10 years old in case of a *Haemophilus influenzae* infection. **New York City medium** contains lincomycin to inhibit Gram positive cocci, amphotericin B to inhibit yeasts, and colistin and trimethoprim to inhibit Gram negative bacilli, and is designed to grow only pathogenic *Neisseria*. However, yeasts and *Enterococcus faecalis* often grow.

Gardnerella vaginalis agar contains nalidixic acid to inhibit staphylococci, amphotericin B to inhibit yeasts and gentamicin to inhibit Gram negative bacilli, and grows *Gardnerella vaginalis*, streptococci and *Lactobacillus*.

MacConkey agar grows Gram negative bacilli and *Enterococcus faecalis*. **Blood agar + vitamin K** will grow all anaerobes. The use of a metronidazole disc on the plate will help to distinguish true anaerobes (nearly all sensitive to metronidazole) from facultative anaerobes (resistant to metronidazole). Gram negative anaerobes can be cultured on **blood agar + vancomycin and kanamycin**. Vancomycin inhibits Gram positives and kanamycin facultative aerobic Gram negatives. *Candida albicans* will grow and *Enterococcus faecalis* will sometimes grow on aged media.