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# Editorial

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We are aware of the fact that Diabetic Foot is one of the major clinical ailments. Treatment of Diabetic Foot requires immense precision and knowledge of the wound conditions. Diabetic patients are at greater risk than the general population to infections, particularly foot infections. Fifteen to 25% of diabetic patients develop a foot ulcer at some time during their life. The pathophysiological mechanisms of diabetic foot infections are still a subject of controversy. Mini Review section will elucidate the basics of this disease and eventually the general Diabetic Foot management procedures.

Under Current Trends, different types of antiseptics are discussed. Most importantly many of the antiseptics are not that effective today as they used to be earlier. So different areas of concern are being discussed in this section.

A small document on The Novel Laureate Elizabeth H. Blackburn is presented in the In Profile section who won 2009- Nobel Prize in Physiology or Medicine for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase.

*Haemophilus* species will be discussed in the Bug of the month segment. *Haemophilus* is a genus of Gram-negative, pleomorphic, coccobacilli bacteria belonging to the Pasteurellaceae family. While *Haemophilus* bacteria are typically small coccobacilli, they are categorized as pleomorphic bacteria because of the wide range of shapes they occasionally assume.

Did You Know section will familiarize with the CMV infection in transplant recipients. Since 1995, many new developments in diagnostic technologies have occurred, and new concepts, such as the indirect effects of CMV, have been recognized. Therefore, the aim of this report is to update and expand the published definitions of CMV, taking into account current knowledge. The definitions have been developed primarily for application to transplant recipients, but they can also be applied to other immune-compromised individuals.

Blood Agar, Hemolysis, and Hemolytic Reactions is explained in the section Best Practices. Blood agar is a nutrient culture medium that is enriched with whole blood and used for the growth of certain strains of bacteria. Blood agar consists of a basal medium such as TSA (Tryptone Soya Agar) enriched with 5% defibrinated sheep blood or in some locations, horse blood. This is the most commonly used medium, and supports the growth of most of the common fastidious organisms, as well as, all of the less fastidious organisms.

JHS team thanks all our readers for their support and contribution in making this journal a success. Feedback and suggestions are always invited.

# Diabetic Foot Management

**Question 1: Definition of diabetic foot infections**

**1a) What is the definition of diabetic foot infection and what are its clinical presentations?**

Infection is defined by invasion of the tissues with proliferation of micro-organisms causing tissue damage with or without an associated inflammatory response by the host. Diabetic foot infections are generally secondary to a skin wound. **The diagnosis of diabetic foot infection is clinical. However, infection must be distinguished from bacterial colonization**, a physiological phenomenon occurring all over the skin and related to minimally virulent aerobic and anaerobic bacteria derived from the skin flora, endogenous flora or the environment. This phenomenon can be modified in diabetes mellitus, with a polymorphic appearance and growth of more virulent bacteria such as *Staphylococcus aureus* or *Streptococcus pyogenes*. The progression to infection occurs as a result of multiple factors related to the characteristics of the wound, the pathogenic bacteria and the host. The diagnosis of **infection** is based on the presence of at least two of the following signs: swelling, induration, erythema around the lesion, local tenderness or pain, local warmth or presence of pus. The severity of infection is assessed according to the International Consensus on the Diabetic Foot classification system (Table 1A).

Table 1A - International Consensus on the Diabetic Foot classification of foot wound infections

| Grade | Description |
|---|---|
| Grade 1 | No symptoms, no signs of infection |
| Grade 2 | Lesion only involving the skin (without involvement of deeper tissues nor Systemic signs) with at least two of the following signs:<br>• local warmth<br>• erythema > 0.5 - 2 cm around the ulcer<br>• local tenderness or pain<br>• local swelling or induration<br>• purulent discharge (thick, opaque to white or sanguineous secretion)<br>Other causes of inflammation of the skin must be eliminated (for example: trauma, gout, acute Charcot foot, fracture, thrombosis, venous stasis) |
| Grade 3 | Erythema > 2 cm and one of the findings described above<br>or<br>Infection involving structures deeper than skin and subcutaneous tissue, such as deep abscess, osteomyelitis, septic arthritis or fasciitis<br>There must not be any systemic inflammatory response (see grade 4) |
| Grade 4 | Any foot infection, in the presence of a systemic inflammatory response manifested by at least two of the following characteristics:<br>• temperature > 38 °C or < 36 °C<br>• pulse > 90 bpm<br>• respiratory rate > 20 per min<br>• $PaCO_2$ < 32mmHg<br>• leukocytes > 12,000 or < 4000 per $mm^3$<br>• 10% of immature (band) forms |

**Superficial infections** involve tissue layers above the superficial fascia and present in the form of acute bacterial cellulitis. **Deep infections** involve the superficial fascia, muscles or bones and joints. **Cellulitis** is a bacterial infection of the subdermis. The clinical features are characterized by local signs (erythema, initially around the lesions and then diffuse). Hyperthermia, ascending lymphangitis and regional lymphadenopathy are sometimes absent in diabetic patients. **Necrotizing cellulitis** is characterized by tissue necrosis of the subdermis and then the dermis. **Necrotizing fasciitis** corresponds to involvement of the superficial fascia, presenting in the form of sloughing of the skin and a violaceous color of the integument without pus or abscess. Rapid deterioration of the general status, development of renal failure, sudden extension of the lesions, cutaneous sensory loss (difficult to demonstrate in patients with diabetic neuropathy) and the presence of skin detachment constitute warning signs of necrotizing fasciitis. **Wet gangrene** is defined by blackish necrotic tissues. It is rapidly progressive with skin detachment and grayish pus with a nauseating odor, and can lead to rapid deterioration of the patient's general status with sepsis, metabolic disorders and renal failure. Purulent collections can present in the form of **abscess** (collected form) or **phlegmon** (circumscribed by the tissues) in the soft tissues of the foot, or even the leg that may sometimes be difficult to demonstrate clinically and may require the use of imaging examinations.

**1b) What are the pathophysiological mechanisms of diabetic foot infections?**

Diabetic patients are at greater risk than the general population to infections, particularly foot infections. Fifteen to 25% of diabetic patients develop a foot ulcer at some time during their life and 40–80% of these ulcers become infected. The pathophysiological mechanisms of diabetic foot infections are still a subject of controversy. The various hypotheses proposed include:
● a **deficiency of cell-mediated immune mechanisms** accentuated by hyperglycemia that can alter leukocyte functions, ● the harmful effects of **neuropathy** and **excessive pressure** on the wound, ● the **chronic nature** of the lesion, ● **hypoxia**, due to a poor local perfusion and accentuated by the host's hypermetabolic state and microbial cellular metabolism. Hypoxia promotes anaerobic subcutaneous infections and decreases the bactericidal activity of neutrophils, ● **arterial disease** decreasing the blood supply to the wound and consequently the influx of endogenous and exogenous factors (antibiotics) involved in the fight against infection, ● the particular **anatomy of the foot**, divided into several compartments, explaining the rapid spread of infection.

**1c) What clinical classifications are available to guide the management of diabetic foot infections?**

Many wound classifications have been proposed. The University of Texas classification (UT classification) is easy to use and should now be used as the **reference wound classification**. It comprises four grades according to depth and four stages according to the presence or absence of infection and/or arterial disease (Table 1B). A complementary classification of wound infections has been defined by the **International Consensus on the Diabetic Foot** (Table 1A). This classification comprises four grades, from grade 1 (no infection) to grade 4 (severe sepsis).

Table 1B

| | Grade 0 Completely epithelialized lesion | Grade 1 Superficial wound | Grade 2 Wound penetrating to tendon or capsule | Grade 3 Wound penetrating to bone or joint |
|---|---|---|---|---|
| Stage A Not infected Not ischemic | 0A (0%) | 1A (0%) | 2A (0%) | 3A (0%) |
| Stage B Infected | 0B (12.5%) | 1B (8.5%) | 2B (28.6%) | 3B (92%) |
| Stage C Ischemic | 0C (25%) | 1C (20%) | 2C (25%) | 3C (100%) |
| Stage D Infected Ischemic | 0D (50%) | 1D (50%) | 2D (100%) | 3D (100%) |

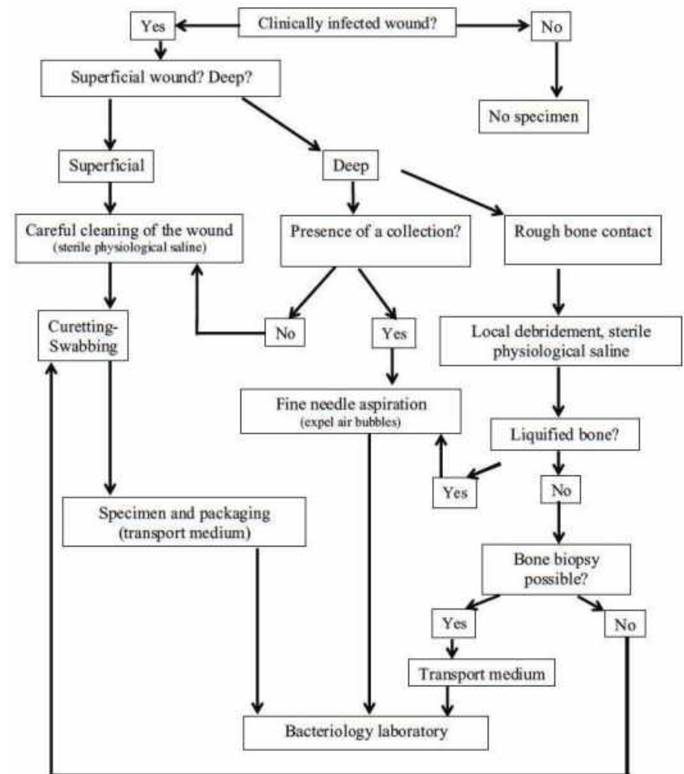
**Question 2: How to document acute diabetic foot infection?**

**2a) How to obtain reliable microbiological data?**
**2a1) What are the indications for bacteriological specimens?**
**Bacteriological specimens are only indicated in the case of clinically confirmed infection**, starting at grade 2 of the International Consensus classification. Bacteriological specimens should not be obtained systematically from wounds with no clinical signs of infection.

**2a2) What are the methods of microbiological isolation?**
Protocols designed jointly by clinicians and microbiologists are essential to obtain clinically useful results. The objective is to obtain isolation and identification of the micro-organism(s) responsible for the infection from a specimen, while avoiding contamination by the commensal flora that colonizes the skin. **No consensus has been reached concerning the best technique for microbiological isolation**. Before taking any specimen, the wound must be prepared. Debridement with a sterile curette or scalpel is essential. The wound must then be cleaned with gauze soaked in sterile physiological saline. Antiseptics can be used, but they must be eliminated by sterile physiological saline before taking the specimen. Fig. 1 summarizes the choice of specimens to be performed as a function of the type of wound.
Repeated specimens should be taken in the event of an unfavorable course or when the patient presents severe sepsis. Specimens must be sent to the microbiology laboratory **as rapidly as possible** (collaboration between clinicians, nurses and couriers), in **transport medium**. Specimens must be **inoculated** on conventional media and incubated at 37 °C. For specimens derived from deep tissues and aspirations, cultures must also be performed under anaerobic conditions. There is a poor correlation between the results of Gram stain and the results of culture of tissue biopsies.

**2a3) Interpretation of the results and epidemiology**
**Interpretation of the results** must take into account the conditions of collection of the specimen, the specimen transport time and transport conditions and the type of bacteria isolated. Firstline treatment should disregard the least virulent or commensal bacteria (coagulase-negative staphylococci, Corynebacteria, *Pseudomonas aeruginosa*, enterococci). There is no 100% reliable microbiological method to distinguish between pathogenic and nonpathogenic micro-organisms at the present time. When there is a doubt, specimens must be repeated and these bacteria will be taken into consideration when they are isolated on several occasions or when the patient's septic state is worrisome.
**Gram-positive aerobic bacteria** are the most frequent microorganisms; in this group, *S. aureus* is the micro-organism most frequently isolated. **Gram-negative aerobic bacteria**, essentially *Enterobacteriaceae*, are generally detected in chronic or previously treated infections. *P. aeruginosa* is frequently isolated after a long stay in hospital or when the wound is treated by moist dressings. **Strict anaerobic bacteria** are often associated with aerobic bacteria. The ecology of multiresistant bacteria must be taken into account, especially Methicillin-resistant *Staphylococcus aureus* (MRSA) which constitutes a very serious problem, although isolation of MRSA is not synonymous with increased virulence. Other bacteria must also be taken into account: *Enterobacteriaceae* resistant to third-generation cephalosporins, multiresistant *P. aeruginosa* and other environmental bacteria.

Fig.1-Flow chart of the specimens to be taken as a function of the type of wounds identified in a diabetic subject




**2b) What is the value of laboratory examinations?**
**No laboratory marker** is sufficiently sensitive and specific to confirm the diagnosis of infection or colonization of a diabetic foot wound. Laboratory markers are often misleading, even in the case of severe lesions.

**Question 3: Apart from microbiological factors, what other risk factors should be investigated?**

**3a) Mechanical factors**
Diabetic neuropathy predisposes to the development of foot deformities and gait abnormalities with the appearance of pressure zones and reactive hyperkeratosis. Continued walking induces the appearance of a zone of inflammatory detachment underneath the zone of hyperkeratosis, leading to mal performance that penetrates into deeper planes and predisposes to the development of infection. The main mechanical factor worsening foot wounds is therefore **continued weightbearing**. Other mechanical factors have also been identified: poorly fitting shoes, nail diseases, acquired deformities (hallux valgus or quintus varus), oedema (predisposing to poor distal arterial perfusion) and prolonged bed-rest. These mechanical risk factors must be identified and eradicated.

**3b) Peripheral vascular disease (PVD)**
**Evaluation of the underlying vascular state is essential in any diabetic patient with an infected wound**. This evaluation is based on clinical examination and complementary investigations:
**3b1) Clinical examination** must look for signs of intermittent claudication, rest pain, that may often not be experienced by the patient due to diabetic neuropathy, with inspection of the foot, auscultation of arteries and palpation of pedal pulses.

**3b2) Complementary investigations**

**a. Ankle-Brachial Index (ABI) must be systematically determined in all diabetic patients**. It corresponds to the ratio between systolic blood pressure measured at the ankle and in the arm (brachial). The ABI may reveal PVD in a number of asymptomatic patients. Normal values are between 0.90 and 1.30. An ABI < 0.90 confirms the diagnosis of PVD. Interpretation of the ABI may be limited by medial sclerosis of ankle arteries which makes the arteries poorly compressible or incompressible, thereby falsely raising the systolic pressure. An ABI > 1.30 is indicative of incompressibility.

**b. Arterial Doppler examination of the lower limbs** is highly recommended in diabetic patients over the age of 40 years and/or who have suffered from diabetes for more than 20 years and/or in the presence of known coronary artery disease or atheroma of the supra-aortic vessels. **This examination must be systematically performed in the case of diabetic foot infection**. B-mode ultrasound identifies stenoses and occlusions, analyses the arterial wall and measures the external diameter of the artery at the site of the stenosis and at a presumably healthy site. Doppler studies provide hemodynamic analysis of flow at the stenosis and in the distal runoff. **Arteriography remains the reference examination** for the anatomical evaluation of PVD and to guide revascularization. **MR angiography** and **CT angiography** (performed without direct arterial injection and without injection of iodinated contrast agent for MR angiography) can be alternatives to arteriography of the lower limbs to evaluate the lesions, especially distal and calcified lesions.

**c. Other examinations**

**Toe blood pressure (TBP),** which can almost always be measured in diabetic patients, is indicated in the case of neuropathy associated with medial arterial sclerosis, when ABI is 1.30. PVD is defined by a 50 mmHg difference between ankle systolic pressure and TBP or by a toe/brachial systolic pressure index < 0.55. A TBP less than 30 mmHg corresponds to critical ischemia and a revascularization procedure must be envisaged.

**Transcutaneous partial pressure of oxygen (TcPO2)** is an index of the severity of skin ischemia and the probability of spontaneous healing even in the presence of medial sclerosis. The normal value of TcPO2 measured on the dorsum of the foot is about 50 mmHg in diabetic patients. For a TcPO2 greater than 30 mmHg, healing is possible in more than 90% of cases. A value less than 20–30 mmHg indicates critical ischemia with a healing rate < 30%. It is falsely lowered in the case of edema of the dorsum of the foot or infection. As a value > 30 mmHg confirms the absence of severe ischemia in a case of diabetic foot infection, **this examination must be performed in the presence of arterial disease**.

**Question 4: What treatment modalities are available?**

**4a) What is the value of a multidisciplinary approach?**

Diabetic foot is a complex disease requiring global management of the patient and not only the foot. A **multidisciplinary approach** is essential and requires good coordination between all health care professionals involved.

**4b) Which strategies should be implemented?**

**4b1) The role of blood glucose control**

There are many arguments in support of **maintaining blood glucose as close to normal as possible**. Insulin therapy is usually required to ensure blood glucose control.

**4b2) The importance of mechanical off-loading**

Complete and permanent off-loading of the wound must be ensured as strictly as possible. Various modalities are available: bed rest, wheelchair (keeping the affected leg horizontal), removable or non-removable casts. The patient's strict compliance with off-loading, tolerance and the condition of the offloading device must be closely monitored.

**4b3) Medical debridement**

**Mechanical debridement** consists of excising necrotic soft tissues, devitalized and contaminated tissues and slough, leaving only healthy tissue in order to promote wound healing. The presence of arterial disease must be eliminated before performing any debridement procedure. In predominantly neuropathic ulcers, debridement must be continued until healthy tissue is reached, but in ischemic ulcers, debridement must be performed very cautiously and must be limited to a simple drainage. Ideally, debridement should be performed after or during revascularization. Debridement allows complete visualization of the wound, exposure of any extensions, better drainage of exudates and collection of deep bacteriological specimens and it also promotes healing. It must always be performed before application of any topical agents and must be repeated as often as necessary.

**4b4) Antiseptics and topical antibiotics**

**Topical antibiotics have no place in the topical treatment of infected foot wounds in diabetic patients. However a topical antiseptic with novel molecule like PHMB (poly-hexa-methylene-biguanide) shows challenging results.**

**4b5) Dressings**

No consensus has been reached concerning the type of dressing to be used use on infected diabetic foot wounds. The principle of daily dressings to allow close surveillance of the infected wound is generally accepted. In the case of cellulitis, the edges of the inflammatory zone must be marked with a felt-tip pen to follow the course. Adhesive or occlusive dressings must not be used on infected wounds. The dressing must be adapted to the volume of exudate. Regardless of the type of dressing applied, detailed wound care protocols must be established and the course of healing must be objectively documented by regular measurement of the wound as well as photographs.

**4b6) Control of edema**

It has been shown that reduction of edema increases the healing rate of debrided diabetic foot infections.

**4b7) Tetanus vaccine status must be systematically verified**

**4b8) Other treatments**

**Hyperbaric oxygen therapy and growth factors** are not currently recommended as treatment for diabetic foot infections. Hyperbaric oxygen therapy can be considered in the case of severe arterial disease (critical ischemia) not suitable for revascularization.

**4c) What surgical strategies are available?**

**4c1) Revascularization procedures** Revascularization procedures have two main objectives: to ensure salvage of a limb when viability is compromised by severe ischemia and to allow healing of ulcers.

**In the case of severe (critical) ischemia** associated with signs of infection, coldness of the foot, pallor, absent pulses, presence of

necrosis and suggestive signs on vascular investigations (ankle blood pressure < 50 mmHg or TcPO2 < 30 mmHg or TBP < 30 mmHg), revascularization must be systematically considered. The treatment of infection (off-loading, debridement, antibiotic therapy) must be started immediately and revascularization must be considered once the infection has been controlled. In the case of surgical exposure, the revascularization procedure should be performed as soon as possible to avoid extension of infection, absence of healing, or even life-threatening deterioration. Ideally, the revascularization should be performed at the same time as the debridement procedure.

**In the case of more moderate ischemia**, a less severe clinical situation and less unfavorable vascular investigations (ankle blood pressure > 70 mmHg, TcPO2 > 30 mmHg and TBP > 50 mmHg), revascularization can be deferred and proposed secondarily, especially in the case of delayed healing despite control of infection and well conducted medical treatment.

**In every case,** it is essential to evaluate the patient's arterial status to assess the need for a revascularization procedure, which could reduce healing time and reoperations.

The **criteria for revascularization** take into account: the patient's general state (operability), the potential for ulcer healing, the quality of the arterial distal runoff and the site of the lesions (arteriography or possibly MR angiography in patients withrenal failure).

The **indications for revascularization** depend on the level of the lesions. Aorto-iliac lesions are treated by angioplasty or bypass graft. Femoropopliteal or tibial lesions should preferably be treated by angioplasty, which do not prevent the possibility of secondary bypass grafts. Multisegmental lesions, the most frequent situation, require a combination of angioplasty and bypass grafts.

**Distal bypass grafts** have transformed the prognosis of serious trophic disorders of the diabetic foot and are not performed sufficiently frequently. They must be performed only after infection has been controlled, preferably with venous material or allografts.

**Lumbar sympathectomy** is not indicated in the treatment of diabetic PVD with or without infected lesions.

**4c2) Orthopaedic surgery**

**Conservative surgery** can be considered in two circumstances:

**Emergency**, in the case of limb-threatening or life-threatening infection, abscess complicated by a compartment syndrome or necrosis, or necrotizing cellulitis. Emergency surgery must be as conservative as possible. Amputations, even minor, must be exceptional.

**Deferred**, in the absence of improvement in response to well conducted medical treatment. This procedure must be performed after vascular assessment and revascularization, if necessary, and must be as conservative as possible.

**Amputation surgery** sometimes still remains the only option in the case of severe, deep infection, especially in a context of ischemia. The choice of the **level of amputation** depends on the vascular status, while taking every effort to preserve heel weightbearing with the prosthesis. No amputation must be performed without first performing complementary vascular investigations.

**Question 5: What are the specificities of osteomyelitis of the diabetic foot?**

**5a) What is the definition of osteomyelitis of the diabetic foot?**

**Bone infection is frequent in diabetic patients, present in 30–80% of cases depending on the severity of the infection**. It may consist of isolated osteomyelitis, especially of the toes and calcaneus, or more often bone and joint infection, while isolated septic arthritis is rare. Bone or joint infection generally occurs by contiguous spread from a skin wound, while a hematogenous origin of osteomyelitis or osteoarthritis of the foot is exceptional in diabetic patients.

**5b) What are the signs suggestive of osteomyelitis of the foot in a diabetic?**

Osteomyelitis must be suspected in the following cases: resistance to treatment, recurrent infection of an ulcer, especially when it is localized over a bony prominence, unfavorable or persistent course despite optimal management and a satisfactory arterial blood supply. Other clinical signs are also in favor of osteomyelitis:

**Rough bone contact** when probing with a sterile, blunt metal probe introduced through the ulcer, although the value of this procedure has recently been questioned;

Bone exposure; the **edematous, erythematous "sausage**" appearance of a toe or abnormal mobility of a toe are also suggestive of bone and joint infection.

**6a) Detection of diabetic patients with a high risk of foot problems**

This consists of identifying risk factors (history of ulceration or amputation, sensory loss of the foot demonstrated by the monofilament test, PVD and foot deformities). These risk factors identify patients according to their level of risk, as defined in the International Consensus classification whose predictive value has been demonstrated by a prospective study.

**6b) Prevention measures**

**6b1) Education**

**Patient education is essential** right from grade 1 and must include the patient's family, comprising practical and adapted advice (awareness of sensory loss and its consequences, awareness of poor blood supply and its consequences, highrisk situations, self-examination of the feet, atraumatic footwear and hygiene and maintenance of the feet (nails, hyperkeratosis, fungal infections).

**Nurse education** must emphasize the importance of regular examination of the feet of diabetic patients, scoring of the risk of foot problems, setting up of preventive strategies based on patient education and foot care.

**6b2) Other measures**

Podiatric care (removal of hyperkeratoses and nail care), good quality shoes, fashioning of orthopaedic shoes and adapted orthoses are essential, as these disorders are responsible for ulceration of the diabetic foot.

**References**

Management of diabetic foot infections, Organized by the Société de Pathologie Infectieuse de Langue rançaise (SPILF) with the participation of the following scientific societies: Association de Langue Française pour l'Étude du Diabète et des Maladies Métaboliques (ALFEDIAM), Société Française de Chirurgie Vasculaire, Société Française de Microbiologie, Collège Français de Pathologie Vasculaire, Médecine et maladies infectieuses 37 (2007) 14–25.

# Antiseptics and Areas of concern

**Alcohols**

Although several alcohols have been shown to be effective antimicrobials, ethyl alcohol (ethanol), isopropyl alcohol (isopropanol, propan-2-ol) and *n*-propanol are the most widely used. Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria (including mycobacteria), viruses, and fungi but are not sporicidal. They are, however, known to inhibit sporulation and spore germination, but this effect is reversible. Because of the lack of sporicidal activity, alcohols are not recommended for sterilization but are widely used for both hard-surface disinfection and skin antisepsis. Lower concentrations may also be used as preservatives and to potentiate the activity of other biocides. Many alcohol products include low levels of other biocides (in particular chlorhexidine), which remain on the skin following evaporation of the alcohol, or excipients (including emollients), which decrease the evaporation time of the alcohol and can significantly increase product efficacy. In general, isopropyl alcohol is considered slightly more efficacious against bacteria and ethyl alcohol is more potent against viruses; however, this is dependent on the concentrations of both the active agent and the test microorganism. For example, isopropyl alcohol has greater lipophilic properties than ethyl alcohol and is less active against hydrophilic viruses (e.g., poliovirus). Generally, the antimicrobial activity of alcohols is significantly lower at concentrations below 50% and is optimal in the 60 to 90% range. Little is known about the specific mode of action of alcohols, but based on the increased efficacy in the presence of water; it is generally believed that they cause membrane damage and rapid denaturation of proteins, with subsequent interference with metabolism and cell lysis. This is supported by specific reports of denaturation of *Escherichia coli* dehydrogenases and an increased lag phase in *Enterobacter aerogenes*, speculated to be due to inhibition of metabolism required for rapid cell division.

**Anilides**

The anilides have been investigated primarily for use as antiseptics, but they are rarely used in the clinic. Triclocarban (TCC; 3,4,49-triclorocarbanilide) is the most extensively studied in this series and is used mostly in consumer soaps and deodorants. TCC is particularly active against gram-positive bacteria but significantly less active against gram-negative bacteria and fungi and lacks appreciable substantivity (persistency) for the skin. The anilides are thought to act by adsorbing to and destroying the semipermeable character of the cytoplasmic membrane, leading to cell death.

**Chlorhexidine**

Chlorhexidine is probably the most widely used biocide in antiseptic products, in particular in handwashing and oral products but also as a disinfectant and preservative. This is due in particular to its broad-spectrum efficacy, substantivity for the skin, and low irritation. Of note, irritability has been described and in many cases may be product specific. Despite the advantages of chlorhexidine, its activity is pH dependent and is greatly reduced in the presence of organic matter. A considerable amount of research has been undertaken on the mechanism of the antimicrobial action of this important bisbiguanide, although most of the attention has been devoted to the way in which it inactivates nonsporulating bacteria.
Nevertheless, sufficient data are now available to examine its sporostatic and mycobacteriostatic action, its effects on yeasts and protozoa, and its antiviral activity. Chlorhexidine is a bactericidal agent. Its interaction and uptake by bacteria were studied initially by Hugo et al., who found that the uptake of chlorhexidine by *E. coli* and *S. aureus* was very rapid and depended on the chlorhexidine concentration and pH. More recently, by using [14C] chlorhexidine gluconate, the uptake by bacteria and yeasts was shown to be extremely rapid, with a maximum effect occurring within 20s. Damage to the outer cell layers takes place but is insufficient to induce lysis or cell death. The agent then crosses the cell wall or outer membrane, presumably by passive diffusion, and subsequently attacks the bacterial cytoplasmic or inner membrane or the yeast plasma membrane. In yeasts, chlorhexidine "partitions" into the cell wall, plasma membrane, and cytoplasm of cells. Damage to the delicate semipermeable membrane is followed by leakage of intracellular constituents, which can be measured by appropriate techniques. Leakage is not per se responsible for cellular inactivation but is a consequence of cell death. High concentrations of chlorhexidine cause coagulation of intracellular constituents. As a result, the cytoplasm becomes congealed, with a consequent reduction in leakage, so that there is a biphasic effect on membrane permeability. An initial high rate of leakage rises as the concentration of chlorhexidine increases, but leakage is reduced at higher biocide concentrations because of the coagulation of the cytosol. Chlorhexidine was claimed by Harold et al. to be an inhibitor of both membrane-bound and soluble ATPase as well as of net K1 uptake in *Enterococcus faecalis*. However, only high biguanide concentrations inhibit membrane-bound ATPase, which suggests that the enzyme is not a primary target for chlorhexidine action. Although chlorhexidine collapses the membrane potential, it is membrane disruption rather than ATPase inactivation that is associated with its lethal effects. The effects of chlorhexidine on yeast cells are probably similar to those previously described for bacteria. Chlorhexidine has a biphasic effect on protoplast lysis, with reduced lysis at higher biguanide concentrations. Furthermore, in whole cells, the yeast cell wall may have some effect in limiting the uptake of the biguanide. The findings presented here and elsewhere demonstrate an effect on the fungal plasma membrane but with significant actions elsewhere in the cell. Increasing concentrations of chlorhexidine (up to 25 mg/ml) induce progressive lysis of *Saccharomyces cerevisiae* protoplasts, but higher biguanide concentrations result in reduced lysis. Work to date suggests that chlorhexidine has a similar effect on the trophozoites of *Acanthameoba castellanii*, with the cysts being less sensitive. Furr reviewed the effects of chlorhexidine and other biocides on *Acanthameoba* and showed that membrane damage in these protozoa is a significant factor in their inactivation. Mycobacteria are generally highly resistant to chlorhexidine. Little is known about the uptake of chlorhexidine (and other antiseptics and disinfectants) by mycobacteria and on the biochemical changes that occur in the treated cells. Since the MICs for some mycobacteria are on the order of those for chlorhexidine-sensitive, gram-positive cocci, the inhibitory effects of chlorhexidine on mycobacteria may not be dissimilar to those on susceptible bacteria. *Mycobacterium avium-intracellulare*is considerably more resistant than other mycobacteria. Chlorhexidine is not sporicidal. Even high concentrations of the bisbiguanide do not affect the viability of *Bacillus* spores at ambient temperatures, although a marked sporicidal effect is achieved at elevated temperatures. Presumably, sufficient changes occur in the spore structure to permit an

increased uptake of the biguanide, although this has yet to be shown experimentally. Little is known about the uptake of chlorhexidine by bacterial spores, although coatless forms take up more of the compound than do "normal" spores. Chlorhexidine has little effect on the germination of bacterial spores but inhibits outgrowth. The reason for its lack of effect on the former process but its significant activity against the latter is unclear. It could, however, be reflected in the relative uptake of chlorhexidine, since germinating cells take up much less of the bisbiguanide than do outgrowing forms. Binding sites could thus be reduced in number or masked in germinating cells. The antiviral activity of chlorhexidine is variable. Studies with different types of bacteriophages have shown that chlorhexidine has no effect on MS2 or K coliphages. High concentrations also failed to inactivate *Pseudomonas aeruginosa* phage F116 and had no effect on phage DNA within the capsid or on phage proteins; the transduction process was more sensitive to chlorhexidine and other biocides than was the phage itself. This substantiated an earlier finding that chlorhexidine bound poorly to F116 particles. Chlorhexidine is not always considered a particularly effective antiviral agent, and its activity is restricted to the lipid-enveloped viruses. Chlorhexidine does not inactivate nonenveloped viruses such as rotavirus, HAV, or poliovirus. Its activity was found by Ranganathan to be restricted to the nucleic acid core or the outer coat, although it is likely that the latter would be a more important target site.

**Alexidine.**

Alexidine differs chemically from chlorhexidine in possessing ethylhexyl end groups. Alexidine is more rapidly bactericidal and produces a significantly faster alteration in bactericidal permeability. Studies with mixed-lipid and pure phospholipid vesicles demonstrate that, unlike chlorhexidine, alexidine produces lipid phase separation and domain formation. It has been proposed that the nature of the ethylhexyl end group in alexidine, as opposed to the chlorophenol one in chlorhexidine, might influence the ability of a biguanide to produce lipid domains in the cytoplasmic membrane.

**Polymeric biguanide** (PHMB).

**Polymeric biguanide** is a hetero disperse mixture of polyhexamethylene biguanides (PHMB) with a molecular weight of approximately 3,000. Earlier Polymeric biguanides have found use as general disinfecting agents in the food industry and, very successfully, for the disinfection of swimming pools. But presently it is found to be very effective antiseptic for acute as well as chronic wounds. This is active against gram-positive and gram-negative bacteria and also sporicidal. PHMB is a membrane-active agent that also impairs the integrity of the outer membrane of gram-negative bacteria, although the membrane may also act as a permeability barrier. Activity of PHMB increases on a weight basis with increasing levels of polymerization, which has been linked to enhanced inner membrane perturbation. Unlike chlorhexidine but similar to alexidine, PHMB causes domain formation of the acidic phospholipids of the cytoplasmic membrane. Permeability changes ensue, and there is believed to be an altered function of some membrane-associated enzymes. The proposed sequence of events during its interaction with the cell envelope of *E. coli* is as follows: (i) there is rapid attraction with strong and specific adsorption to phosphate-containing compounds; (ii) the integrity of the outer membrane is impaired, and PHMB is attracted to the inner membrane; (iii) binding of PHMB to phospholipids occurs, with an increase in inner membrane permeability (K1 loss) accompanied by bacteriostasis; and (iv) complete loss of membrane function follows, with precipitation of intracellular constituents and a bactericidal effect.

**Iodine and iodophors.**

Although less reactive than chlorine, iodine is rapidly bactericidal, fungicidal, tuberculocidal and virucidal. Although aqueous or alcoholic (tincture) solutions of iodine have been used for 150 years as antiseptics, they are associated with irritation and excessive staining. In addition, aqueous solutions are generally unstable; in solution, at least seven iodine species are present in a complex equilibrium, with molecular iodine (I2) being primarily responsible for antimictrobial efficacy. These problems were overcome by the development of iodophors ("iodine carriers" or "iodine-releasing agents"); the most widely used are povidone-iodine and poloxamer-iodine in both antiseptics and disinfectants. Iodophors are complexes of iodine and a solubilizing agent or carrier, which acts as a reservoir of the active "free" iodine. Although germicidal activity is maintained, iodophors are considered less active against certain fungi and spores than are tinctures. Similar to chlorine, the antimicrobial action of iodine is rapid, even at low concentrations, but the exact mode of action is unknown. Iodine rapidly penetrates into microorganisms and attacks key groups of proteins (in particular the freesulfur amino acids cysteine and methionine, nucleotides, and fatty acids, which culminates in cell death. Less is known about the antiviral action of iodine, but nonlipid viruses and parvoviruses are less sensitive than lipid enveloped viruses. Similarly to bacteria, it is likely that iodine attacks the surface proteins of enveloped viruses, but they may also destabilize membrane fatty acids by reacting with unsaturated carbon bonds.

**Silver Compounds**

In one form or another, silver and its compounds have long been used as antimicrobial agents. The most important silver compound currently in use is silver sulfadiazine (AgSD), although silver metal, silver acetate, silver nitrate, and silver protein, all of which have antimicrobial properties, are listed in *Martindale*, *The Extra Pharmacopoeia*. In recent years, silver compounds have been used to prevent the infection of burns and some eye infections and to destroy warts.

**Silver nitrate.**

The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfydryl,™SH) groups, although other target sites remain a possibility. Liau et al demonstrated that amino acids such as cysteine and other compounds such as sodium thioglycolate containing thiol groups neutralized the activity of silver nitrate against *P. aeruginosa*. By contrast, amino acids containing disulfide (SS) bonds, non-sulfurcontaining amino acids, and sulfur-containing compounds such as cystathione, cysteic acid, L-methionine, taurine, sodium bisulfite, and sodium thiosulfate were all unable to neutralize Ag1 activity. These and other findings imply that interaction of Ag1 with thiol groups in enzymes and proteins plays an essential role in bacterial inactivation, although other cellular components may be involved. Hydrogen bonding, the effects of hydrogen bond-breaking agents, and the specificity of Ag1 for thiol groups were discussed in greater detail by Russell and Hugo. Virucidal properties might also be explained by binding to ™SH groups. Lukens proposed that silver salts and other heavy metals such as copper act by binding to key functional groups of fungal enzymes. Ag1 causes the release of K1 ions from microorganisms; the microbial plasma or cytoplasmic membrane, with which is associated many important enzymes, is an important target site for Ag1 activity. In addition to its effects on enzymes, Ag1 produces other changes in microorganisms. Silver nitrate causes marked inhibition of growth of *Cryptococcus neoformans* and is deposited in the vacuole and cell wall as granules. Ag1 inhibits cell division

and damages the cell envelope and contents of *P. aeruginosa*. Bacterial cells increase in size, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers all exhibit structural abnormalities, although without any blebs (protuberances). Finally, the Ag1 ion interacts with nucleic acids; it interacts preferentially with the bases in DNA rather than with the phosphate groups, although the significance of this in terms of its lethal action is unclear.

**Silver sulfadiazine.**

AgSD is essentially a combination of two antibacterial agents, Ag1 and sulfadiazine (SD). The question whether the antibacterial effect of AgSD arises predominantly from only one of the compounds or via a synergistic interaction has been posed repeatedly. AgSD has a broad spectrum of activity and, unlike silver nitrate, produces surface and membrane blebs in susceptible (but not resistant) bacteria. AgSD binds to cell components, including DNA. Based on a chemical analysis, Fox proposed a polymeric structure of AgSD composed of six silver atoms bonding to six SD molecules by linkage of the silver atoms to the nitrogens of the SD pyrimidine ring. Bacterial inhibition would then presumably be achieved when silver binds to sufficient base pairs in the DNA helix, thereby inhibiting transcription. Similarly, its antiphage properties have been ascribed to the fact that AgSD binds to phage DNA. Clearly, the precise mechanism of action of AgSD has yet to be solved.

**Bis-Phenols**

The bis-phenols are hydroxy-halogenated derivatives of two phenolic groups connected by various bridges. In general, they exhibit broad-spectrum efficacy but have little activity against *P. aeruginosa* and molds and are sporostatic toward bacterial spores. Triclosan and hexachlorophane are the most widely used biocides in this group, especially in antiseptic soaps and hand rinses. Both compounds have been shown to have cumulative and persistent effects on the skin.

**Triclosan**

Triclosan (2,4,49-trichloro-29-hydroxydiphenyl ether; Irgasan DP 300) exhibits particular activity against grampositive bacteria. Its efficacy against gram-negative bacteria and yeasts can be significantly enhanced by formulation effects. For example, triclosan in combination with EDTA caused increased permeability of the outer membrane. Reports have also suggested that in addition to its antibacterial properties, triclosan may have anti-inflammatory activity. The specific mode of action of triclosan is unknown, but it has been suggested that the primary effects are on the cytoplasmic membrane. In studies with *E. coli*, triclosan at subinhibitory concentrations inhibited the uptake of essential nutrients, while higher, bactericidal concentrations resulted in the rapid release of cellular components and cell death. Studies with a divalent-ion-dependent *E. coli* triclosan mutant for which the triclosan MIC was 10-fold greater than that for a wild-type strain showed no significant differences in total envelope protein profiles but did show significant differences in envelope fatty acids. Specifically, a prominent 14:1 fatty acid was absent in the resistant strain, and there were minor differences in other fatty acid species. It was proposed that divalent ions and fatty acids may adsorb and limit the permeability of triclosan to its site of action. Minor changes in fatty acid profiles were recently found in both *E. coli* and *S. aureus* strains for which the triclosan MICs were elevated; however, the MBCs were not affected, suggesting, as for other phenols, that the cumulative effects on multiple targets contribute to the bactericidal activity.

**Hexachlorophene**

Hexachlorophene (hexachlorophane; 2,29-dihydroxy-3, 5, 6, 39, 59, 69-hexachlorodiphenylmethane) is another bis-phenol whose mode of action has been extensively studied. The primary action of hexachlorophene, based on studies with *Bacillus megatherium*, is to inhibit the membranebound part of the electron transport chain, and the other effects noted above are secondary ones that occur only at high concentrations. It induces leakage, causes protoplast lysis, and inhibits respiration. The threshold concentration for the bactericidal activity of hexachlorphene is 10 mg/ml (dry weight), but peak leakage occurs at concentrations higher than 50 mg/ml and cytological changes occur above 30 mg/ml. Furthermore, hexachlorophene is bactericidal at 0°C despite causing little leakage at this temperature. Despite the broad-spectrum efficacy of hexachlorophene, concerns about toxicity, in particular in neonates, have meant that its use in antiseptic products has been limited.

**Halophenols**

Chloroxylenol (4-chloro-3,5-dimethylphenol; *p*-chloro-*m*-xylenol) is the key halophenol used in antiseptic or disinfectant formulations. Chloroxylenol is bactericidal, but *P. aeruginosa* and many molds are highly resistant. Surprisingly, its mechanism of action has been little studied despite its widespread use over many years. Because of its phenolic nature, it would be expected to have an effect on microbial membranes.

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**Elizabeth H. Blackburn**
Born: 26 November 1948, Hobart, Tasmania, Australia
Affiliation at the time of the award: University of California, San Francisco, CA, USA

**Honors & Awards**

- 2000 - American Association for Cancer Research-G.H.A. Clowes Memorial Award
- 2000 - American Cancer Society Medal of Honor
- 2000 - Elected, Fellow of the American Association for the Advancement of Science
- 2003 - 26th Annual Bristol-Myers Squibb Award for Distinguished Achievement in Cancer Research
- 2005 - Kirk A. Landon-American Association for Cancer Research Prize for Basic Cancer Research
- 2005 - Benjamin Franklin Medal in Life Science
- 2005 - New York Academy of Medicine Medal
- 2006 - Fifth annual Wiley Prize in Biomedical Sciences
- 2006 - The 25th Meyenburg Prize
- 2006 - Elected, American Philosophical Society
- 2007 - TIME 100 Most Influential People in the World
- 2008 - L'Oreal UNESCO For Women In Science Award
- 2008 - Weizmann Women & Science Award: Weizmann Institute of Science
- 2009 - Nobel Prize in Physiology or Medicine for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase

**Biography**

Dr. Elizabeth H. Blackburn, Morris Herztein Professor of Biology and Physiology in the Department of Biochemistry and Biophysics at the University of California, San Francisco, is a leader in the area of telomere and telomerase research.

She discovered the molecular nature of telomeres - the ends of eukaryotic chromosomes that serve as protective caps essential for preserving the genetic information - and the ribonucleoprotein enzyme, telomerase. Blackburn and her research team at the University of California, San Francisco are working with various cells including human cells, with the goal of understanding telomerase and telomere biology.

She was born in the small city of Hobart in Tasmania, Australia, in 1948. Her parents were family physicians. Her grandfather and great grandfather on my mother's side were geologists. Her uncle and aunt (father's sister and mother's brother) were also both family physicians, who moved to England, married there and permanently settled there to practice medicine and raise their families.

She was the second child of eventually seven siblings. She spent Her first 4 years living in the tiny town of Snug, by the sea near Hobart. Curious about animals, She would pick up ants in her backyard and jellyfish on the beach. Then her family moved to Launceston, a town in northern Tasmania. Her first house, at 120 Abbott Street, was a one-storied, verandahed house of typical Australian suburban architecture. She started kindergarten at a girls' school, Broadland House Girls Grammar School in Launceston.

Perhaps arising from a fascination with animals, biology seemed the most interesting of sciences to her as a child. She was captivated by both the visual impact of science through science books written for young people, and an idea of the romance and nobility of the scientific quest. This latter was especially engendered by the biography of Marie Curie, written by her daughter, which she read as a child. By the time she was in her late teens it was clear to her that she wanted to do science. She was educated at Broadland House Girls Grammar School, and received a generally excellent education. However, physics was not offered, so she took physics classes offered in the evenings at the local public high school. She loved playing piano, and even at one time wistfully hoped that she might become a musician. Fortunately she was quite realistic about this, because she recognized that she was competent rather than greatly talented at piano playing, so she went in the direction of science.

Her family moved, after some family disruptions, to the city of Melbourne, Australia, in time for her to complete her last year of high school at University High School. There she gained the confidence that she needed to apply for the undergraduate science degree at the University of Melbourne.

Blackburn earned her B.Sc. (1970) and M.Sc. (1972) degrees from the University of Melbourne in Australia, and her Ph.D. (1975) from the University of Cambridge in England. She did her postdoctoral work in Molecular and Cellular Biology from 1975 to 1977 at Yale.

In 1978, Blackburn joined the faculty at the University of California at Berkeley in the Department of Molecular Biology. In 1990, she joined the Department of Microbiology and Immunology at UC San Francisco, where she served as Department Chair from 1993 to 1999. Blackburn is currently a faculty member in Department of Biochemistry and Biophysics at UCSF. She is also a Non-Resident Fellow of the Salk Institute.

Throughout her career, Blackburn has been honored by her peers as the recipient of many prestigious awards. She was elected President of the American Society for Cell Biology for the year 1998. Blackburn is an elected Fellow of the American Academy of Arts and Sciences (1991), the Royal Society of London (1992), the American Academy of Microbiology (1993), and the American Association for the Advancement of Science (2000).

She was elected Foreign Associate of the National Academy of Sciences in 1993, and was elected as a Member of the Institute of Medicine in 2000. She was awarded the Albert Lasker Medical Research Award in Basic Medical Research (2006). In 2007 she was named one of TIME Magazine's 100 Most influential People and she is the 2008 North American Laureate for L'Oreal-UNESCO For Women in Science.

In 2009, Dr. Blackburn was awarded the Nobel Prize in Physiology or Medicine.

# Enjoy the humour

### Home Cooked Date

Alex met his close friend Archie for guidance, and narrated to him that he recently met a girl of his dreams. He asked Archie's advice as to how should he proceed now!

So wise Archie said, "Well send her roses, and on the name card invite her for a home-cooked meal."

Alex liked the idea, so he invited the woman.

Next day after the dinner Archie called Alex and asked him how did the home-cooked dinner go.

Alex cried, "It flopped miserably."

Archie asked, "Why? Didn't the girl come at your house?"

Alex said, "She came, but she refused to cook and left angrily!"

### Cricketers mindset: "DO vs DIE".

Ganguly: Do or die.

Sehwag: Do before you die.

Dravid: Do until they die.

Tendulkar: Do that will never die...

Laxman: Do when everyone else die.

Yuvraj: Do, die, reborn, do, die, reborn (repeat)....

Dhoni: Do everything before luck die.

### Awesome Answer

In a meeting VP said the recent attrition rate was so high. It was the quarter end and work was huge. "If we do not complete the work on time, we need to be paying heavy penalty" said the VP.

The VP then turned to the manger and told "Hey take how much ever resources you want recruit or take them from other departments, but complete the work in another 25 days. Take people and complete it man.

To this the sweet manager humbly replied "Sir! Give me one wife and nine months and I shall show you results, Don't give me nine wives and one month, I cannot do anything." Everyone looked at him blank!

The VP did not have anything else to say and just walked away........

## Proud Facts about INDIA

India never invaded any country in her last 100000 years of history.

Chess was invented in India.

Algebra, Trigonometry and Calculus are studies, which originated in India.

The 'Place Value System' and the 'Decimal System' were developed in India in 100 B.C.

The game of Snakes & Ladders was created by the 13th century poet saint Gyandev. It was originally called 'Mokshapat'. The ladders in the game represented virtues and the snakes indicated vices.

India has the largest number of Post Offices in the world.

Bhaskaracharya rightly calculated the time taken by the earth to orbit the Sun hundreds of years before the astronomer Smart. According to his calculation, the time taken by the Earth to orbit the Sun was 365.258756484 days.

The value of "pi" was first calculated by the Indian Mathematician Budhayana, and he explained the concept of what is known as the Pythagorean Theorem. He discovered this in the 6th century, long before the European mathematicians.

The Baily Bridge is the highest bridge in the world. It is located in the Ladakh valley between the Dras and Suru rivers in the Himalayan mountains. It was built by the Indian Army in August 1982.

India exports software to 90 countries.

Martial Arts were first created in India, and later spread to Asia by Buddhist missionaries.

With 395 Articles and eight Schedules, the Indian Constitution is the largest written constitution in the world.

## In Praise of India

1. **Will Durant, American historian:** "India was the motherland of our race, and Sanskrit the mother of Europe's languages: she was the mother of our philosophy; mother, through the Arabs, of much of our mathematics; mother, through the Buddha, of the ideals embodied in Christianity; mother, through the village community, of self-government and democracy. Mother India is in many ways the mother of us all".

2. **Mark Twain, American author:** "India is, the cradle of the human race, the birthplace of human speech, the mother of history, the grandmother of legend, and the great grandmother of tradition. Our most valuable and most instructive materials in the history of man are treasured up in India only."

3. **Albert Einstein, American scientist:** "We owe a lot to the Indians, who taught us how to count, without which no worthwhile scientific discovery could have been made."

4. **Max Mueller, German scholar:** If I were asked under what sky the human mind has most fully developed some of its choicest gifts, has most deeply pondered on the greatest problems of life, and has found solutions, I should point to India.

5. **Romain Rolland, French scholar :** "If there is one place on the face of earth where all the dreams of living men have found a home from the very earliest days when man began the dream of existence, it is India."

6. **Henry David Thoreau, American Thinker & Author:** Whenever I have read any part of the Vedas, I have felt that some unearthly and unknown light illuminated me. In the great teaching of the Vedas, there is no touch of sectarianism. It is of all ages, climbs, and nationalities and is the royal road for the attainment of the Great Knowledge. When I read it, I feel that I am under the spangled heavens of a summer night.

# Haemophilus species

## Introduction

*Haemophilus* is a genus of Gram-negative, pleomorphic, coccobacilli bacteria belonging to the Pasteurellaceae family. While *Haemophilus* bacteria are typically small coccobacilli, they are categorized as pleomorphic bacteria because of the wide range of shapes they occasionally assume.

The genus *Haemophilus* includes a number of species that cause a wide variety of infections but share a common morphology and a requirement for blood-derived factors during growth that has given the genus its name. *Haemophilus influenzae*, the major pathogen, can be separated into encapsulated or typable strains, of which there are seven types (a through f including e') based on the antigenic structure of the capsular polysaccharide, and unencapsulated or nontypable strains. Type b *H influenzae* is by far the most virulent organism in this group, commonly causing bloodstream invasion and meningitis in children younger than 2 years. Nontypable strains are frequent causes of respiratory tract disease in infants, children, and adults.

Other *Haemophilus* species cause disease less frequently. *Haemophilus parainfluenzae* sometimes causes pneumonia or bacterial endocarditis. *Haemophilus ducreyi* causes chancroid. *Haemophilus aphrophilus* is a member of the normal flora of the mouth and occasionally causes bacterial endocarditis. *Haemophilus aegyptius*, which causes conjunctivitis and Brazilian purpuric fever, and *Haemophilus haemolyticus* used to be separated on the basis of their ability to agglutinate or lyse red blood cells, but both are now included among the nontypable *H influenzae* strains.

## Pathogenesis

The pathogenesis of *H influenzae* infections is not completely understood, but the presence of the type b polysaccharide capsule is a major factor in virulence. Encapsulated organisms can penetrate the epithelium of the nasopharynx and invade blood capillaries directly. Nontypable strains are less invasive, but they, as well as typable strains, induce an inflammatory response that causes disease; production of exotoxins is not thought to play a role in pathogenicity. Nontypable *H influenzae* strains colonize the nasopharynx of most normal individuals, but type b *H influenzae* strains are found in only 1 to 2 percent of normal children. Outbreaks of type b infection occur, especially in nurseries and child care centers; prophylactic administration of antibiotics may be used. Vaccination with type b polysaccharide appears to be effective in preventing infection, and vaccines are now available for routine use.

## Structure, Classification, and Antigenic Types

*Haemophilus* species are Gram-negative coccobacilli that share common ultrastructural features with other Gram-negative bacilli. Their cell walls contain lipooligosaccharide, which resembles the lipopolysaccharide of Gram-negative bacilli but has shorter side chains (hence the designation oligosaccharide rather than polysaccharide). *Haemophilus* species have generally been thought not to make toxins or other extracellular products that account for their ability to produce infection. These organisms require hemin (factor X) and/or nicotinamide adenine dinucleotide (NAD+) (factor V) for growth. Whereas NAD+ is released into the medium by red blood cells and is available to the bacteria in blood agar, hemin is bound to red blood cells and is not released into the medium unless the cells are broken up, as in chocolate agar. *Haemophilus influenzae* requires both factors X and V; accordingly, it grows on chocolate agar but not on blood agar, although it may appear on a blood agar plate as tiny satellite colonies around the colonies of other bacteria that have lysed red blood cells. *Haemophilus parainfluenzae* requires only factor V and therefore is able to grow on blood agar (however, recent reports suggest that many isolates identified as *H parainfluenzae* actually are *H paraphrophilus*). The long-prevailing notion that *H ducreyi* grows only in clotted rabbit blood has been dispelled by recent studies that show slow growth of this organism in Mueller-Hinton agar containing 5 percent sheep blood. All *Haemophilus* species grow more readily in an atmosphere enriched with $CO_2$; *H ducreyi* and some nontypable *H influenzae* strains will not form visible colonies on culture plates unless grown in $CO_2$-enriched atmosphere.

*Haemophilus influenzae* strains are classified as either serotypable (if they display a capsular polysaccharide antigen) or nontypable (if they lack a capsule). The word "type" as applied to *H influenzae* refers to this serotyping scheme. There are six generally recognized types: a, b, c, d, e, and f. A seventh type has been designated e' because its polysaccharide is closely related to that of type e. Antiserum to type e' *H influenzae* is not routinely available. These types may be identified by an agglutination reaction that uses antisera raised in rabbits; with this method, however, cross-reactions with somatic antigens may cause nontypable strains to be designated erroneously as typable. This kind of error is eliminated by using counterimmuno-electrophoresis, in which migration under an electric current removes somatic (protein) antigens from the reaction, leaving only capsular polysaccharides to react with antibody.

The presence of a polyribosyl ribitol phosphate (PRP) capsule is an important virulence factor: it renders type b *H influenzae* resistant to phagocytosis by polymorphonuclear leukocytes in the absence of specific anticapsular antibody. Susceptibility to the bactericidal effect of serum depends on the presence of antibodies to a number of antigenic sites, including the lipooligosaccharide or outer membrane proteins designated as P1 and P2. Type b *H influenzae* is plainly the most virulent of the *Haemophilus* species; 95 percent of bloodstream and meningeal *Haemophilus* infections in children are due to this organisms. In contrast, in adults, nontypable strains of *H influenzae* are the most common cause of *Haemophilus* infection, presumably because most adults have acquired antibody to PRP.

The relative place of *H influenzae* biogroup aegyptius, the cause of Brazilian purpuric fever, remains to be determined. Most, but not all, strains that cause this syndrome contain a unique 24 megadalton plasmid and a 79 kilodalton outer membrane protein,

either or both of which may mediate virulence. These organisms also have at least some of the genetic material that codes for encapsulation of type b *H influenzae*.

By using a series of biochemical reactions, *H influenzae* also may be classified into six biotypes designated I through VI. Most type b *H influenzae* strains fall into biotypes I or II, whereas most non typable *H influenzae* strains fall into biotypes II through VI. Several interesting clinical correlations have recently emerged. Biotype I isolates appear to have a predilection for causing pneumonia. Nearly all genital isolates, as well as bloodstream isolates from infected neonates or from women with puerperal sepsis, are biotype IV. In addition, biotype III, which agglutinates red blood cells in vitro and includes *H influenzae* biogroup aegyptius, has been implicated as a common cause of conjunctivitis. There is no explanation for these clinically observed associations.

**Clinical Manifestations**

*Haemophilus* species cause a variety of clinical syndromes. Until the implementation of widespread vaccination programs, type b *H influenzae* was the most common cause of meningitis in children between the ages of 6 months and 2 years. In this situation, headache is followed rapidly by development of a stiff neck, with progression to coma and, in the absence of treatment, death. Emergent treatment reduces the incidence of, but does not eliminate, sequelae such as deafness and learning disabilities. Type b *H influenzae* also causes cellulitis and epiglottitis, a condition in which the epiglottitis becomes inflamed and swells, closing off the upper airway. Suffocation can be prevented in some cases only by performing a tracheostomy. Nontypable *H influenzae* strains commonly cause infection of the middle ear (otitis media), which manifests as an earache with fever in babies and young children. In adults, these organisms cause bronchitis and pneumonia, especially if some underlying disease of the bronchi and lungs is present. Nontypable *H influenzae* strains also commonly cause acute or chronic sinusitis in patients of all ages. Chancroid is a venereal disease caused by *H ducreyi*. Lesions that resemble a syphilitic chancre result from sexual contact with an infected individual; they are usually found on the genitals. Unlike syphilitic chancres, the lesions are painful and are associated with a remarkable degree of swelling of lymph nodes in the inguinal area.

**Host Defenses**

For many years it was believed that bactericidal antibody directed against PRP capsule of type b *H influenzae* was entirely responsible for host resistance to infection. However, more recent studies have stressed a role for antibody to somatic antigens as well. For example, antibody to PRP can often be detected in the sera of children on admission to the hospital with sepsis due to type b *H influenzae*. In addition, adsorption of immune serum with PRP alone does not remove its protective capabilities, whereas adsorption with whole organisms does. Finally, immunization with ribosomes is protective in animal models of infection. Separation of the outer membrane of type b *H influenzae* into its many protein constituents by polyacrylamide gel electrophoresis (PAGE) combined with analysis of antibody responses during infection has suggested that antibody to any of a number of individual membrane proteins may be associated with immunity. Bactericidal antibodies that react with individual outer membrane proteins or with lipooligosaccharide constituents have been identified. These findings support, on a molecular basis, the potential importance of antibody to noncapsular antigens in immunity to type b *H influenzae* infection. Opsonizing antibody may also play a role in protection and may be directed against PRP or somatic constituents.

Recent studies of nontypable *H influenzae* strains have shown that bactericidal antibody to outer membrane proteins develops in infants in response to otitis media caused by these organisms. Normal adults generally have both bactericidal and opsonizing antibodies directed against nontypable *H influenzae*. Although levels of opsonizing antibody may be low in adults who develop acute nontypable *H influenzae* infection, substantial levels of bactericidal activity are present in serum at the time infection is diagnosed. It is not clear why this should occur. In some instances a blocking effect by secretory IgA in bronchial secretions might be responsible. Alternatively, the extensive structural damage to the bronchi and lungs that predisposes to serious nontypable *H influenzae* infection may allow proliferation of the bacteria unchecked by normal serum defense mechanisms.

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# CMV in Transplant Recipients

A first set of CMV definitions was developed and published as part of the proceedings of the 4th International CMV Conference in Paris in 1993. These definitions were updated at the 5th International CMV Conference in Stockholm in 1995 and have since been used in many published studies. Since 1995, many new developments in diagnostic technologies have occurred, and new concepts, such as the indirect effects of CMV, have been recognized. Therefore, the aim of this report is to update and expand the published definitions of CMV, taking into account current knowledge. The definitions have been developed primarily for application to transplant recipients, but they can also be applied to other immune-compromised individuals. We recognize that these definitions are, in part, unsuitable for application to HIV-infected patients.

**CMV Infection:** "CMV infection" is defined as isolation of the CMV virus or detection of viral proteins or nucleic acid in any body fluid or tissue specimen. It is recommended that both the source of the specimens tested (e.g., plasma, serum, whole blood, peripheral blood leukocytes, CSF, urine, or tissue) and the diagnostic method used be described clearly.

**CMV Detection in Blood:** Several specific definitions for CMV detection in blood are recommended. ***Viremia.*** "Viremia" is defined as the isolation of CMV by culture that involves the use of either standard or shell vial techniques. ***Antigenemia.*** "Antigenemia" is defined as the detection of CMV pp65 in leukocytes. ***DNAemia.*** "DNAemia" is defined as the detection of DNA in samples of plasma, whole blood, and isolated peripheral blood leukocytes or in buffy-coat specimens. There are several techniques available for the detection of DNAemia, including PCR-based techniques, hybrid capture, and branched-chain DNA analysis. The tests can be either qualitative or quantitative. For quantitative tests, the technique used for quantification should be specified. It is recommended that true quantitative, rather than semi-quantitative, techniques be used to measure the virus load. ***RNAemia.*** "RNAemia" is defined as the detection of RNA (e.g., by nucleic acid sequence–based amplification or noncommercial reverse transcriptase–PCR) in samples of plasma, whole blood, or isolated peripheral blood leukocytes or in buffy-coat specimens.

**Primary CMV Infection:** "Primary CMV infection" is defined as the detection of CMV infection in an individual previously found to be CMV seronegative. The appearance of de novo specific antibodies in a seronegative patient may also be acceptable for the diagnosis of CMV, provided that passive transfer of antibodies via immunoglobulin or blood products can be excluded.

**Recurrent Infection:** "Recurrent infection" is defined as new detection of CMV infection in a patient who has had previously documented infection and who has not had virus detected for an interval of at least 4 weeks during active surveillance. Recurrent infection may result from reactivation of latent virus (endogenous) or reinfection (exogenous). ***Reinfection.*** "Reinfection" is defined as detection of a CMV strain that is distinct from the strain that was the cause of the patient's original infection. ***Reactivation.*** Reactivation is assumed if the 2 strains are found to be indistinguishable either by sequencing specific regions of the viral genome or by using a variety of molecular techniques that examine genes known to be polymorphic.

**CMV End-Organ Disease:** A general problem involves how to report copathogens together with CMV. Each pathogen's relative importance is frequently difficult to assess, and, therefore, it is important that the presence of copathogens be reported clearly. ***Pneumonia.*** "CMV pneumonia" is defined by the presence of signs and/or symptoms of pulmonary disease combined with the detection of CMV in bronchoalveolar lavage fluid or lung tissue samples. ***Gastrointestinal disease.*** "CMV gastrointestinal disease" is defined by identification of a combination of clinical symptoms from the upper or lower gastrointestinal tract, findings of macroscopic mucosal lesions on endoscopy, and demonstration of CMV in a gastrointestinal tract biopsy specimen. ***Hepatitis.*** "CMV hepatitis" is defined by findings of elevated bilirubin and/or enzyme levels during liver function testing, absence of any other documented cause of hepatitis, and detection of CMV infection (by culture, psychopathologic testing, immunohistochemical analysis, or in situ hybridization) in a liver biopsy specimen. ***CNS disease.*** "CNS disease" is defined by the identification of CNS symptoms together with the detection of CMV in CSF samples, by culture or PCR, or in brain biopsy specimens, by culture, histopathologic testing, immunohistochemical analysis, or in situ hybridization. ***Nephritis.*** "CMV nephritis" can be defined by the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of histologic features of CMV infection in a kidney biopsy specimen obtained from a patient with renal dysfunction. ***Cystitis.*** "CMV cystitis" is defined by the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of conventional histologic features of CMV infection in a bladder biopsy specimen obtained from a patient with cystitis. ***Myocarditis.*** "CMV myocarditis" is defined by the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of conventional histologic features of CMV infection in a heart biopsy specimen obtained from a patient with myocarditis. ***Pancreatitis.*** The definition of CMV pancreatitis requires the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of conventional histologic features of CMV infection in a pancreatic biopsy specimen obtained from a patient with pancreatitis. ***CMV syndrome.*** The term "CMV syndrome" should be avoided. Although it is recognized that CMV can cause the combination of fever and bone marrow suppression that is usually used to define the disease entity, the same symptoms can have several other different causes in stem cell transplant recipients, including such viral infections as human herpesvirus 6 (HHV-6), possibly human herpesvirus 7, and adenovirus. At present, the minimum requirements for its definition are the documented presence of fever (temperature, 138°C) for at least 2 days within a 4-day period, the presence of neutropenia or thrombocytopenia, and the detection of CMV in blood. ***CMV-associated graft failure.*** Several publications have suggested that CMV can induce graft failure after stem cell transplantation. It is difficult to define CMV-associated graft failure, because several other possible causes of graft failure exist, including graft rejection, relapse of hematologic disease, drug toxicity, and infection with other viruses (e.g., HHV-6, Epstein-Barr virus, and parvovirus).

**INDIRECT EFFECTS:** In addition to directly causing end-organ diseases, CMV is associated statistically with graft rejection, accelerated atherosclerosis, and fungal or bacterial superinfection, which collectively are known as the "indirect effects" of CMV.

**Acute Graft Rejection:** Evidence from several cohort studies shows that CMV infection is associated with an increased risk of acute graft rejection. This has been shown for recipients of heart, lung, kidney, and liver transplants.

**Transplantation Atherosclerosis:** After heart transplantation, CMV infection was associated with greater incidence and greater severity of coronary atherosclerosis and a higher rate of graft loss in CMV-seropositive hearttransplant recipients [4]. In a rat model, CMV infection accelerated cardiac allograft atherosclerosis. This effect could be prevented by administration of prophylactic ganciclovir.

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# Blood Agar, Hemolysis, and Hemolytic Reactions

Blood agar is a nutrient culture medium that is enriched with whole blood and used for the growth of certain strains of bacteria.

In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis. Blood Agar Base media are specified in standard method procedures for food testing.

Blood agar consists of a basal medium such as TSA (Tryptone Soya Agar) enriched with 5% defibrinated sheep blood or in some locations, horse blood. This is the most commonly used medium, and supports the growth of most of the common fastidious organisms, as well as, all of the less fastidious organisms.

Blood agar is a solid growth medium that contains red blood cells. The medium is used to detect bacteria that produce enzymes to break apart the blood cells. This process is also termed hemolysis. The degree to which the blood cells are hemolyzed is used to distinguish bacteria from one another.

The blood agar medium is prepared in a two-step process. First, a number of ingredients are added to water, including heart infusion, peptone, and sodium chloride. This solution is sterilized. Following sterilization , a known amount of sterile blood is added. The blood can be from rabbit or sheep. Rabbit blood is preferred if the target bacterium is from the group known as group A *Streptococcus*. Sheep blood is preferred if the target bacterium is *Haemophilus parahaemolyticus*.

Blood agar is a rich food source for bacteria. So, it can be used for primary culturing, that is, as a means of obtaining as wide a range of bacterial growth from a sample as possible. It is typically not used for this purpose, however, due to the expense of the medium. Other, less expensive agars will do the same thing. What blood agar is uniquely suited for is the determination of hemolysis.

Hemolysis is the break down of the membrane of red blood cells by a bacterial protein known as hemolysin, which causes the release of hemoglobin from the red blood cell. Many types of bacterial posses hemolytic proteins. These proteins are thought to act by integrating into the membrane of the red blood cell and either punching a hole through the membrane or disrupting the structure of the membrane in some other way. The exact molecular details of hemolysin action is still unresolved.

The blood used in the agar is also treated beforehand to remove a molecule called fibrin, which participates in the clotting of blood. The absence of fibrin ensures that clotting of the blood does not occur in the agar, which could interfere with the visual detection of the hemolytic reactions.

When streaked on Blood Agar, many species of bacteria cause hemolysis – i.e., destruction of the erythrocytes (and hemoglobin) in the medium. Hemolytic reactions are generally classified as alpha, beta or gamma according to the appearance of zones around isolated colonies growing on or in the medium:

**Alpha hemolysis:** The colony is surrounded by a zone of intact but discolored erythrocytes that have a greenish color. This appearance is generally due to the action of peroxide produced by the bacteria. This type of hemolysis represents a partial decomposition of the hemoglobin of the red blood cells. Alpha hemolysis is characteristic of *Streptococcus pneumonia* and so can be used as a diagnostic feature in the identification of the bacterial strain.

When Alpha hemolysis (α-hemolysis) is present the agar under the colonies is dark and greenish. *Streptococcus pneumoniae and a group of oral streptococci (Streptococcus or viridans streptococci) display alpha hemolysis. This is sometimes called green hemolysis* because of the color change in the agar. Other synonymous terms are *incomplete hemolysis* and *partial hemolysis*. Alpha hemolysis is caused by hydrogen peroxide produced by the bacterium, oxidizing hemoglobin to green methemoglobin .

*Streptococcus* is a genus of spherical Gram-positive bacteria belonging to the phylum Firmicutes and the lactic acid bacteria group. Most *streptococci* are oxidase- and catalase-negative, and many are facultative anaerobes. In the medical setting, the most important groups are the alpha-hemolytic streptococci, *S. pneumoniae* and *Streptococcus Viridans*-group.

**Beta hemolysis:** The colony is surrounded by a white or clear zone in which few or no intact erythrocytes are found. This reaction is best seen when the organism is growing under anaerobic conditions. Beta hemolysis is caused by one or more erythrocyte-lysing enzymes called hemolysins.

Beta hemolysis represents a complete breakdown of the hemoglobin of the red blood cells in the vicinity of a bacterial colony. There is a clearing of the agar around a colony. Beta hemolysis is characteristic of *Streptococcus pyogenes* and some strains of *Staphylococcus aureus*.

Beta hemolysis (β-hemolysis), sometimes called *complete hemolysis*, is a complete lysis of red cells in the media around and under the colonies: the area appears lightened (yellow) and transparent. Streptolysin, an exotoxin, is the enzyme produced by the bacteria which causes the complete lysis of red blood cells. There are two types of streptolysin: Streptolysin O (SLO) and streptolysis S (SLS). Streptolysin O is an oxygen-sensitive cytotoxin, secreted by most Group A streptococcus (GAS), and interacts with cholesterol in the membrane of eukaryotic cells (mainly red and white blood cells, macrophages, and platelets), and usually results in β-hemolysis under the surface of blood agar. Streptolysin S is an oxygen-stable cytotoxin also produced by most GAS strains which results in clearing on the surface of blood agar. SLS affects immune cells, including polymorphonuclear leukocytes and lymphocytes, and is thought to prevent the host immune system from clearing infection. *Streptococcus pyogenes , or Group A beta-hemolytic Strep (GAS), displays beta hemolysis.*

Some weakly beta-hemolytic species cause intense beta hemolysis when grown together with a strain of *Staphylococcus* . *This is called the CAMP test . Streptococcus agalactiae displays this property. Clostridium perfringens can be identified presumptively with this test.*

The most important groups of beta-hemolytic streptococci are Lancefield groups A and B (also known as "Group A strep" and "Group B strep"). Beta-hemolytic streptococci are further characterized via the Lancefield serotyping – based on specific carbohydrates in the bacterial cell wall.

**Group A**

S. pyogenes, also known as Group A Streptococcus (GAS), is the causative agent in Group A streptococcal infections, including

streptococcal pharyngitis ("strep throat" AmE), acute rheumatic fever, scarlet fever, acute glomerulonephritis and necrotizing fasciitis. Other *Streptococcus* species may also possess the Group A antigen, but human infections by non-S. pyogenes GAS strains (some S. dysgalactiae subsp. equisimilis and S. anginosus Group strains) appear to be uncommon.

Group A Streptococcus infection is generally diagnosed with a Rapid Strep Test (AmE) or by culture. Rheumatic fever, a disease that affects the joints and heart valves, is a consequence of untreated strep A infection caused not by the bacterium itself. Rheumatic fever is caused by the antibodies created by the immune system to fight off the infection cross-reacting with other proteins in the body. This cross-reaction causes the body to essentially attack itself and leads to the damage above.

**Group B**

*S. agalactiae*, or GBS, causes pneumonia and meningitis in neonates and the elderly, with occasional systemic bacteremia. They can also colonize the intestines and the female reproductive tract, increasing the risk for premature rupture of membranes and transmission to the infant. The American College of Obstetricians and Gynecologists, American Academy of Pediatrics and the Centers for Disease Control recommend all pregnant women between 35 and 37 weeks gestation should be tested for GBS. Women who test positive should be given prophylactic antibiotics during labor, which will usually prevent transmission to the infant.

**Group C**

Includes *S.equi*, which causes strangles in horses, and *S.zooepidemicus* - *S.equi* is a clonal descendent or biovar of the ancestral *S.zooepidemicus* - which causes infections in several species of mammals including cattle and horses.

**Group F**

Group F streptococci were first described in 1934 by Long and Bliss amongst the "minute haemolytic streptococci". They are also known as Streptococcus anginosus (according to the Lancefield classification system) or as members of the *S.milleri* group (according to the European system).

**Group G**

These streptococci are usually but not exclusively beta hemolytic. *Streptococcus canis* is an example of a GGS which is typically found on animals but can cause infection in humans.

**Gamma hemolysis** is simply a synonym for negative hemolysis in which there is no change in the medium surrounding the colony. A blood agar plate displaying gamma hemolysis actually appears brownish. This is a normal reaction of the blood to the growth conditions used (37° C in the presence of carbon dioxide). Gamma hemolysis is a characteristic of *Enterococcus faecalis*.

If an organism does *not* induce hemolysis, it is said to display gamma hemolysis (γ-hemolysis): the agar under and around the colony is unchanged (this is also called *non-hemolytic*). *Enterococcus faecalis (formerly called Group D Strep) displays gamma hemolysis.*

Hemolytic reactions can also display some synergy. That is, the combination of reactions produces a reaction that is stronger than either reaction alone. Certain species of bacteria, such as group B Strep (n example is *Streptococcus agalactiae*) are weakly beta-hemolytic. However, if the bacteria are in close proximity with a strain of *Staphylococcus* the betahemolysins of the two organisms can combine to produce an intense beta hemolytic reaction. This forms the basis of a test called the CAMP test (after the initials of its inventors).

The determination of hemolysis and of the hemolytic reactions is useful in distinguishing different types of bacteria. Subsequent biochemical testing can narrow down the identification even further. For example, a beta hemolytic reaction is indicative of a Streptococcus. Testing of the Streptococcus organisms with bacitracin is often the next step. Bacitracin is an antimicrobial that is produced by the bacterium *Bacillus subtilis*. *Streptococcus pyogenes* strains are almost uniformally sensitive to bacitracin. But other antigenic groups of Streptococcus are not bacitracin sensitive.

**Applications:**

- Blood agar is used to support the growth of fastidious organisms and to determine the type of hemolysis (destruction of red blood cell walls) an organism produces.
- Chocolate agar (CHOC), type of blood agar plate in which the blood cells have been lysed by heating the cells to 56 °C. Chocolate agar is used for growing fastidious (fussy) respiratory bacteria, such as *Haemophilus influenzae*.
- Tryptose Agar is used for cultivating a wide variety of fastidious microorganisms, particularly for isolating Brucella. Tryptose Agar with 5% bovine serum, with or without antibiotics, remains a standard plating medium for the isolation of brucellae. For isolation of Brucella stains from contaminated milk, crystal violet (gentian violet) can be added to Tryptose Agar to suppress gram-positive organisms. Tryptose media can be supplemented with thiamine or citrate for the cultivation and maintenance of fastidious aerobic and facultative microorganisms.

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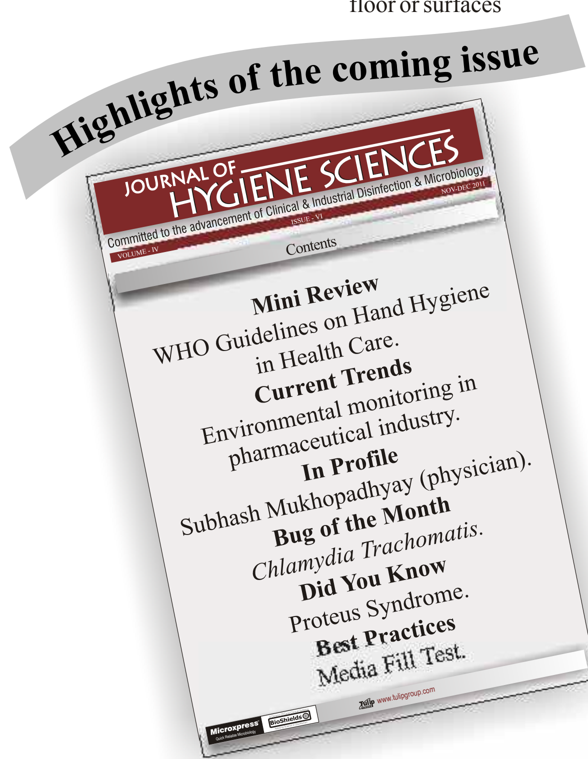


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