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Editorial

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Well to Jump start with this issue we have the 'Mini Review' section giving us a brief on "PHMB". The antiseptic agent polyhexamethylene biguanide (also known as polihexanide or PHMB) has been used for over 60 years in a wide range of applications from swimming pool sanitisers to preservatives in cosmetics and contact lens solutions. PHMB is a fast-acting biguanide compound composed of a synthetic mixture of polymers. The compound is structurally similar to the antimicrobial peptides (AMPs) produced by many cells within the wound, such as keratinocytes and inflammatory neutrophils, where they are thought to help protect against infection.

Our Current Trends section highlights about Fungal Infections. As with other microbial infections, the diagnosis of fungal infections depends upon a combination of clinical observation and laboratory investigation. Laboratory tests can help in establishing or confirming the diagnosis of a fungal infection, in providing objective assessments of response to treatment, and in monitoring resolution of the infection. The successful laboratory diagnosis of fungal infection depends in major part on the collection of appropriate clinical specimens for investigation. It is also dependent on the selection of appropriate microbiological test procedures.

In Profile Scientist – "Antonie van Leeuwenhoek"- somewhat improbable father of microbiology. A moderately educated owner of a textile business, he learned how to make his own unique microscopes which offered unparallelled magnification. Using these microscopes he made a number of crucially important scientific discoveries, including single-celled animals and plants, bacteria, and spermatozoa.

Bug of the month - ***Wuchereria bancrofti*** is a human parasitic roundworm that is the major cause of lymphatic filariasis. It is one of the three parasitic worms, besides *Brugia malayi* and *B. timori* that infect lymphatic system to cause lymphatic filariasis. These filarial worms are spread by a mosquito vector. *W. bancrofti* is the most prevalent among the three and affects over 120 million people, primarily in Central Africa and the Nile delta, South and Central America, and the tropical regions of Asia including southern China and the Pacific. If the infection is left untreated, it can develop into a chronic disease called elephantiasis. In rare conditions it also causes tropical eosinophilia, an asthmatic disease.

Did You Know? In microbiology, McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing.

A microbiology laboratory is a unique environment that requires special practices and containment facilities in order to properly protect persons working with microorganisms. Safety in the laboratory is the primary concern.

All work & no play makes Jack a dull boy! We don't forget that ever. Each issue comes with its own bouquet of jokes, so enjoy……

Our JHS team is thankful to all our readers for their ever increasing appreciation that has served as a reward & motivation for us. Feedback & suggestions are always welcomed.

PHMB: an effective antimicrobial in wound bioburden management

Wound infection results from the complex interaction between an individual's immune system, the condition of the wound and the number and virulence of bacteria present (Thomson and Smith, 1994; Dow, 2001; Dowsett et al, 2004; Stotts, 2004; Best Practice Statement, 2011). Underlying medical problems such as poor blood supply, hypoxia and metabolic disorders are also contributing factors (Hunt and Hopf, 1997). If bacterial species are allowed to flourish, the states of colonisation, critical colonisation, or wound infection, as outlined in the wound infection continuum (Kingsley, 2001; White et al, 2001), will occur. This is not only costly to the patient, but also has serious financial and legal implications for healthcare providers. Reducing the risk of infection through effective management of wound bioburden is thus an essential aspect of wound care (Shultz et al, 2003; World Union of Wound Healing Societies (WUWHS), 2008).

Effects of bioburden

Although there are no clinical studies on the impact of specific microorganisms on the healing process, clinicians agree that infection causes serious delays in healing as a result of the expression of bacterial virulence factors (Schlüter and Konig, 1990; Thomson, 2000). These factors are believed to damage the wound bed in a variety of ways:

- Microorganisms consume nutrients and oxygen required for wound repair
- Protease virulence determinants (e.g. elastase) damage the extracellular matrix
- White cell function is impaired by the release of short-chain fatty acids produced by anaerobes. Endotoxins stimulate production of interleukins: tumor necrosing factor and matrix metalloproteinases (MMPs)
- Free oxygen radical production increases
- Imbalances occur between MMPs and tissue inhibitors of metalloproteinases (TIMPs)
- Fibroblast production is decreased or delayed, collagen disorganised and scar strength decreased.

Additional consequences for the patient may include increased pain and discomfort, inconvenience, and life-threatening illness. Adverse consequences for the healthcare system may be extended hospital stay, heightened risk of litigation and increased treatment costs incurred by extra antibiotic and dressing usage, as well as extra staff costs.

Approaches to bioburden control

Spreading infection in an individual has serious implications for patient wellbeing and acts as a pathogenic reservoir, increasing the risk of cross-contamination. Accurate differential diagnosis and treatment with appropriate systemic antibiotics is essential (European Wound Management Association (EWMA), 2006; WUWHS, 2008). Topical antibiotics, which are linked to bacterial resistance, should be avoided (WUWHS, 2008). Adjuvant topical antimicrobial dressings may be used to help reduce the wound bioburden (EWMA, 2006; WUWHS, 2008).

However, critical colonisation and localised, subclinical infection remain an issue, and are significant contributors to prolonged wound healing (Edwards and Harding, 2004; Warriner and Burrell, 2005). In recent years, topical antimicrobial agents have become the first line of treatment in managing bacterial burden, particularly in chronic wounds (White et al, 2001; Cooper, 2004). Their prophylactic use remains controversial, but can be justified in immunocompromised individuals or where there is a high risk of infection. Current opinion suggests that the ideal antimicrobial is:

- Associated with minimal systemic absorption
- Effective against likely contaminants and pathogens
- Fast-acting, with prolonged residual activity after a single dose
- Inexpensive
- Incapable of promoting bacterial resistance
- Non-carcinogenic and non-teratogenic (i.e. does not cause DNA damage, which could result in carcinoma or foetal abnormality) to host cells
- Non-toxic
- Widely available (Drosou et al, 2003).

Antimicrobial dressings should be capable of bactericidal activity against both planktonic bacteria and those in biofilm colonies. In addition, the active substances must be contained in a delivery system that would normally, although not exclusively, be a contact dressing, which can be left in contact with the wound for 12 hours or more and remain active for the duration of wear time (Best Practice Statement (BPS), 2011).

Until relatively recently, the main antimicrobial compounds used by clinicians in the UK contained silver, iodine, chlorhexidine, and honey. The rapid rise in the availability of antimicrobial products is testament to the growing significance placed on clinical bioburden-control. These are now commercially available in a number of dressing formats. But, despite their widespread use, there is concern regarding their indiscriminate and prolonged application (BPS, 2011). In some cases, there are specific issues relating to their use, and these will be covered throughout the following sections of this article.

Silver

Silver-based products are extensively used in wound care (Klasen 2000a, 2000b; Demling and De Santi, 2001; Clarke, 2003), with skin discolouration (argyria) and irritation being the only visible side effects (White, 2002). It is thought that silver has a number of antimicrobial modes of action (Thurman and Gerba, 1989; Russell and Hugo, 1994). However, questions have been raised over the long-term use of these dressings, especially in infants (Denyer, 2009a; 2009b). Recently, there have been concerns about silver toxicity (Parsons et al, 2005; Burd et al, 2007), and the systemic uptake and deposition of silver in organs have been noted in a number of studies (Wan et al, 1991; Denyer, 2009a; Wang et al, 2009). To date, the pathological consequences of this are unknown. Added to this, there are fears about the emergence

of silver resistance (Percival et al, 2005; Loh et al, 2009). It would seem that, in academic circles at least, questions exist over its continued widespread clinical use. This has been further enhanced by questions about its cost-effectiveness (Bergin and Wraight, 2006; Chaby et al, 2007; Michaels et al, 2009), which in some areas has led to product restrictions.

Iodine

Iodine-based products have been used in wound care for many years. Like all antiseptics, iodine simultaneously affects multiple sites in microbial cells, resulting in cell disruption and death (Cooper, 2007). However, not only have its antimicrobial efficacy and chemical stability been debated, but also its toxicity to host tissues and the ensuing effect on patient comfort (Kramer, 1999; Wilson et al, 2005). It has been found that providone-iodine is not as effective as some other biocides in eradicating *Staphylococcus epidermis* within in vitro biofilms (Presterl et al, 2007). Cadexomer iodine provides sufficient iodine for biofilm suppression without causing significant damage to the host (Akiyama et al, 2004; Rhoads et al, 2008) but pain has been reported as a side effect of its use (Hansson, 1998).

Honey

In recent years, there has been resurgence in interest in honey-based products for bioburden management (White, 2002). The exact mode of action of honey is not yet fully understood. However, it is hyperosmolar and, thus, restricts the availability of environmental water to bacteria and other organisms (Molan, 2001), leading to cell disruption and death. However, this effect is lessened as the honey becomes more diluted by wound exudate (Molan, 1999). A secondary action is the release of hydrogen peroxide as the honey is diluted by exudate (Molan and Betts, 2004). However, some honeys, particularly *Leptospermum* or manuka varieties, have been found to retain their bactericidal properties even without the presence of hydrogen peroxide (Cooper et al, 2002a; 2002b), which is thought to be associated with a phytochemical component (Karayil et al, 1998; Molan, 2002). The antibacterial properties of honey, therefore, vary according to its source.

Chlorhexidine

Chlorhexidine has been used clinically for about 50 years (Russell, 2002). It is active against gram-negative organisms such as *Pseudomonas aeruginosa* and gram-positive organisms such as *Staphylococcus aureus* and *Escherichia coli*, although meticillin-resistant *Staphylococcus aureus (*MRSA) resistance has been recorded (Cookson, 2000). Chlorhexidine appears to be relatively safe, with little effect on the healing process. However, results from studies are insufficient to draw conclusions about its use on open wounds. In addition, there are concerns about the safety of additives frequently used in chlorhexidine-based preparations to modify their handling properties. More human trials need be performed to assess its efficacy and long-term safety (White et al, 2001; Main, 2008).

The dilemma

Careful and objective review of the literature suggests that the use of many antiseptics in wound management must be subject to a risk-benefit assessment of possible local toxicity and beneficial antibacterial action (Brennan and Leaper, 1985). In short, it is advised that, before use, the beneficial antimicrobial effects and bioavailability should be weighed against any possible cellular toxicity (Wilson et al, 2005).

Given the widespread availability of antimicrobial products, factors likely to influence selection include:

- Clinician familiarity
- Availability, cost and reimbursement issues
- Ease of use and implications for pattern of care
- Efficacy and safety (WUWHS, 2008).

As there appears to be concern about the safety and efficacy of commonly used and familiar antimicrobial products, clinicians need to cast the net wider and search for alternative safe, effective and efficient products.

PHMB

The antiseptic agent polyhexamethylene biguanide (also known as polihexanide or PHMB) has been used for over 60 years in a wide range of applications from swimming pool sanitisers to preservatives in cosmetics and contact lens solutions. In Europe, it has been available as a wound irrigation fluid for some time.

PHMB is a fast-acting biguanide compound composed of a synthetic mixture of polymers. The compound is structurally similar to the antimicrobial peptides (AMPs) produced by many cells within the wound, such as keratinocytes and inflammatory neutrophils, where they are thought to help protect against infection (Sorensen et al, 2003; Ousey and McIntosh, 2009). AMPs have a broad spectrum of activity against bacteria, viruses and fungi, inducing cell death by disrupting cell membrane integrity (Ikeda et al, 1983; Ikeda et al, 1984; Moore and Gray, 2007;).

The structural similarities to AMP mean that PHMB can infiltrate bacterial cell membranes and kill bacteria in a similar way (Moore and Gray, 2007). However, PHMB does not interfere with the proteins that make up animal cell membranes. It, therefore, has a specific antimicrobial action that does not affect animal cell integrity. It is thought that, once it has adhered to the target cell membranes, PHMB causes them to leak potassium ions and other dissolved ions from the cytoplasm (Davies et al, 1968; Davies and Field, 1969; Broxton et al, 1984a; Yasuda et al, 2003; Gilbert, 2006), resulting in cell death. PHMB has an effect on both planktonic bacteria and those in biofilms (Seipp et al, 2005; Pietsch and Kraft, 2006; Harbs and Siebert, 2007). Its action on the bacterial cell membrane also means that the efflux pump (a mechanism used by many bacterial cells to remove toxins) is unable to remove the antiseptic, so intracellular bactericidal concentrations are maintained (Kingsley et al, 2009). Once inside the cell, there is evidence that PHMB binds to DNA and other nucleic acids, suggesting it may also damage or inactivate bacterial DNA (Allen et al, 2004).

Studies have shown that PHMB is effective in vitro, while clinical studies indicate it has a broad spectrum of activity, including against human immunodeficiency virus (HIV) (Wérthen et al, 2004; Krebs et al, 2005). Testing has demonstrated that exposure to PHMB causes viral cells to clump together, forming aggregates. This prevents invasion into the host cells, making PHMB a potent antiviral treatment in wound care (Pinto et al, 2009).

However, studies have shown that the product is safe in clinical use. Schnuch et al (2000; 2007) demonstrated that in trials including 3529 patients, skin sensitisation to PHMB is low

(approximately 0.5%), even when the tested concentrations (2.5% and 5%) were 5–10 times that normally used in wound applications. Comparative tests of PHMB's biocompatibility (measurement of an antiseptic agent's activity in relation to its cytotoxicity) against other commonly used therapies have demonstrated its superiority to chlorhexidine, povidone-iodine, triclosan, silver and sulpadiazine (Müller and Kramer, 2008). In addition, no known resistance to PHMB has been reported, most likely owing to its rapid and non-specific bactericidal activity (Moore and Gray, 2007).

PHMB-based wound care

Recently, PHMB has been successfully incorporated into a range of wound products with various formats. These products offer the clinician alternative methods of using PHMB in bioburden management. These products include:

- Solutions and gels (e.g. Prontosan® wound irrigation solution and wound gel (B Braun Medical Ltd), which contains 0.1% solution of PHMB)
- Non-adherent bacterial-barrier products (e.g. the AMD™ range of infection control dressings (Covidien), which are impregnated with 0.2% PHMB. The product range includes Telfa™ AMD non-adherent wound dressings, Kerlix™ AMD gauze dressings, Excilon™ AMD drain intravenous sponges) A new addition is Kendall AMD Antimicrobial Foam, which has a higher percentage of PHMB impregnated 0.5%. These are all class IIb products
- Biocellulose PHMB-donating dressings (e.g. Suprasorb® X+PHMB (Activa Healthcare, a Lohmann & Rauscher company), which incorporates 0.3% PHMB. These are all class III products.

In current classification dressings on the market (EU classification IIb), the PHMB molecule has been chemically bound to the base material, providing it with antimicrobial properties when in contact with wound moisture. The product, therefore, protects against the development of wound infection by decreasing the bacterial load in the dressing and prevents bacterial penetration through the dressing.

In PHMB-donating products (EU classification III), the active component is not chemically bound to the dressing material, and so can be delivered into the wound and periwound tissues. Here, the dressing is a carrier for a wider antimicrobial activity as it donates the PHMB into the wound.

Wound care products incorporating PHMB have been shown to have positive effects on wound healing. In vitro and in vivo studies have shown that, in some of these products, the influence of PHMB:

- Reduces wound pain rapidly and effectively (Daeschlein et al, 2007; Galitz et al, 2009)
- Reduces wound malodour (Daeschlein et al, 2007)
- Increases formation of granulation tissue (Mueller and Krebsbach, 2008)
- Increases keratinocyte and fibroblast activity (Wiegand et al, 2008a)
- Reduces slough within the wound (Mueller and Krebsbach, 2008)
- Reduces MMP-induced periwound breakdown (Cazzaniga et al, 2002; Werthen et al, 2004)
- Helps remove non-viable tissue (Kaehn, 2009).

The success of PHMB has resulted in its recommendations as the primary antimicrobial in many European countries (Dissemond et al, 2010) and has prompted the publication of a UK consensus review (Wounds UK, 2010).

Conclusion

PHMB appears to meet the criteria for an ideal antimicrobial agent, as described by Drosou et al (2003), and is available in presentations that provide clinicians with effective wound-care modalities for most clinical scenarios. Clinical use, both in the UK and the wider healthcare community, has shown PHMB-based wound-care products to be effective options for managing wound colonisation and infection and, so, deserve closer scrutiny.

Laboratory diagnosis of fungal infection

Introduction:

As with other microbial infections, the diagnosis of fungal infections depends upon a combination of clinical observation and laboratory investigation. Superficial fungal infections often produce characteristic lesions which suggest a fungal diagnosis, but it is not unusual to find that the appearance of lesions has been modified and rendered atypical by previous treatment.

In most situations where deep fungal infection is entertained as a diagnosis, the clinical presentation is nonspecific and can be caused by a wide range of infections, underlying illness or complications of treatment. Nor can radiological or other diagnostic imaging methods be relied upon to distinguish fungal infection from other causes of disease.

Laboratory tests can help in establishing or confirming the diagnosis of a fungal infection, in providing objective assessments of response to treatment, and in monitoring resolution of the infection. The successful laboratory diagnosis of fungal infection depends in major part on the collection of appropriate clinical specimens for investigation. It is also dependent on the selection of appropriate microbiological test procedures.

In neutropenic patients and transplant recipients, invasive fungal infection often presents as persistent fever that fails to respond to broad-spectrum antibacterial treatment. The successful management of these patients often depends on the prompt initiation of empirical antifungal treatment without waiting for formal confirmation of the diagnosis. It is essential that these high-risk individuals be subjected to frequent microbiological surveillance for fungal infection.

Collection of specimens

To establish or confirm the diagnosis of suspected fungal infection, it is essential for the clinician to provide the laboratory with adequate specimens for investigation.

Inappropriate collection, storage or processing of specimens can result in a missed diagnosis. Moreover, to ensure that the most appropriate laboratory tests are performed, it is essential for the clinician to indicate that a fungal infection is suspected and to provide sufficient background information.

In addition to specifying the source of the specimen and its time of collection, it is important to provide information on any underlying illness, recent travel or previous residence abroad, any animal contacts and the patient's occupation if considered relevant. This information will help the laboratory to anticipate which fungal pathogens are most liable to be involved and permit the selection of the most appropriate test procedures. In addition, the laboratory ***must*** be informed if there are particular risks associated with the handling of the specimen, for instance if the patient has hepatitis or human immunodeficiency virus (HIV) infection.

With the exception of skin, hair and nails, specimens for mycological examination should be collected into and transported to the laboratory in sterile containers appropriate to the type of material being investigated. All specimen containers should be clearly labelled.

Skin, nails and hair

Skin, nails and hair should be collected into folded squares of black paper (about 10 x 10 cm). The use of paper permits the specimen to dry out, which helps to reduce bacterial contamination, and also provides a convenient means of storing specimens for long periods (12 months or longer). It is often helpful to clean superficial lesions with 70% alcohol prior to sampling as this will improve the chances of detecting fungus on microscopic examination, as well as reducing the likelihood of bacterial contamination of cultures. Prior cleaning is essential if ointments, creams or powders have been applied to the lesion.

Material should be collected from cutaneous lesions by scraping outwards from the margin of the lesion with a blunt scalpel. If there is minimal scaling, it is helpful to use clear adhesive tape, or adhesive skin sampling discs, to remove material for examination. The sellotape strip or disc should be pressed against the lesion, peeled off and placed, adhesive-side-down, on a clean glass microscope slide for transportation to the laboratory.

It is often helpful to use a Wood's light to select infected scalp hairs for laboratory investigation. If none of the hairs give the green fluorescence which is a feature of some forms of dermatophyte scalp infection, a search should be made for lustreless hairs or stumps, and for hairs broken off at follicle mouths. Hairs should be plucked from the scalp with forceps. Cut hairs without roots are unsuitable for mycological investigation because the infection is usually confined near or below the surface of the scalp.

Mucous membranes

Although scrapings from oral lesions are better than swabs for diagnosis of oral infections, the latter are more frequently used, mainly because they are more convenient for transporting material to the laboratory. Swabs should either be moistened with sterile water or saline prior to taking the sample, or sent to the laboratory in transport medium.

For vaginal infections, swabs should be taken from discharge in the vagina and from the lateral vaginal wall. Swabs should be sent to the laboratory in transport medium.

Ear

Scrapings of material from the ear canal are to be preferred, although swabs can also be used.

Eye

Material from a corneal ulcer with a suspected fungal cause should be collected by scraping the ulcer with a sterile platinum spatula. The entire base of the ulcer as well as the edges should be sampled. Because the amount of material that can be obtained will be small, it is best transferred to an agar plate for culture and to a glass slide for microscopic examination at the bedside.

The plate should be marked to indicate the point of inoculation before being sent to the laboratory. Swabs are not suitable for sampling corneal lesions. In patients with suspected fungal endophthalmitis, vitreous humour should be collected whenever possible. Vitreous humour specimens that have been diluted by the irrigating solution should be concentrated by centrifugation before being examined in the laboratory.

Blood

Blood culture should be performed in all cases of suspected deep fungal infection. However, unless specialized techniques or media are used, clinicians should not expect blood cultures taken

for isolation of bacteria to detect fungi other than ***Candida*** species, ***Cryptococcus neoformans*** or ***Trichosporon*** species. Isolation of fungi from blood depends on a number of factors, including the amount of blood sampled, the number of samples collected, and the method of processing. Culture of arterial blood should be considered if venous blood cultures are unsuccessful in a patient with suspected deep mycosis.

In general, ***Cundia*** species are more readily recovered from blood than are dimorphic fungi and moulds. Isolation rates are higher when the medium is vented and aerated, and biphasic media incorporating both agar and broth phases are more effective than broth alone. The chances of successful isolation are increased if multiple samples of blood are collected and larger volumes are cultured.

Cerebrospinal fluid

Cerebrospinal fluid (CSF) specimens of **3-5** ml are ideal, but are often smaller than this. Samples can be centrifuged and the supernatant fluid used for serological tests. The sediment can be cultured, but is also useful for microscopic examination.

Urine

In non-catheterized patients, fresh midstream specimens of urine are adequate for mycological investigation, provided care is taken to ensure that vaginal or perineal infection does not lead to contamination. In infants, suprapubic aspiration is the best method of urine collection.

Urine samples should be processed for microscopic examination and culture, but can also be tested for fungal antigens.

Patients with blastomycosis or cryptococcosis may have prostatic infection, and it is therefore important to collect urine specimens following prostatic massage. The specimen should be centrifuged and the sediment cultured. Other disseminated infections that can be diagnosed on the basis of a positive urine culture include coccidioidomycosis and histoplasmosis.

Other fluids

Chest, abdominal and joint fluids, whether aspirated or drained, should be collected into sterile containers which include a small amount of sterile heparin (diluted 1:1000) to prevent clotting. The specimens should be centrifuged and the sediment cultured. Drain fluid from patients on continuous peritoneal dialysis should be collected in a sterile container without heparin.

Lower respiratory tract specimens

Fresh, early morning samples of sputum are ideal. These should be collected in sterile containers and processed within 2 h of collection. If delay in processing is unavoidable, specimens must be stored at **4°C.** If the patient does not have a productive cough, a sputum sample may be induced by introducing nebulized saline into the bronchial tree. It is recommended that at least three samples of sputum be submitted for microscopic examination and culture whenever a fungal infection is suspected: 24-h collections of sputum are not suitable for mycological investigation.

In immunocompromised patients, the most useful procedures for collection of lower respiratory tract specimens are bronchoalveolar lavage (BAL) or a bronchial wash. These procedures are carried out with a fibreoptic bronchoscope and provide good material for microscopic examination and culture. Specimens should be centrifuged and the sediment examined.

Percutaneous needle biopsies are useful in patients with focal lung disease, in particular those with peripheral lesions which are not accessible to a bronchoscope. Large needles are better than fine needles and the procedure should be carried out under radiological guidance. Specimens should be processed for microscopic examination and culture.

PUS

If possible, swabs should not be used to collect material from draining abscesses or ulcers. If a swab must be used, then material should be taken from as deep as possible within the lesion. Pus from undrained subcutaneous abscesses or sinus tracts should be aspirated with a sterile needle and syringe. If grains are visible in the pus (as in mycetoma), these should be collected. In mycetoma, if the crusts at the opening of sinus tracts are lifted, grains can often be found in the pus underneath.

Bone marrow

These specimens are useful for making the diagnosis in a number of deep fungal infections, including histoplasmosis, cryptococcosis and paracoccidioidomycosis. About **3-5** ml aspirated material should be collected into a sterile container which includes a small amount of sterile heparin (diluted 1:1000).

Tissue

Tissue specimens should be placed in sterile saline and ***not*** in formalin. If possible, material should be obtained from both the middle and the edge of lesions. Total excision of small cutaneous, subcutaneous or mucosal lesions is often possible.

Specimens for serological tests

Serological tests for dimorphic fungal pathogens are much more helpful if paired or sequential specimens are collected. Blood, CSF, urine and other biological fluids for serological testing should be collected into glass or plastic tubes without anticoagulants; 5-10 ml is usually sufficient.

Specimens for antifungal drug level determinations

The concentrations of antifungal drugs are measured for **two** principal reasons: to ensure that adequate drug concentrations are attained and to ensure that concentrations that could cause unpleasant or even harmful side-effects are avoided.

Blood and other biological fluids should be collected into glass or plastic tubes without anticoagulants; 5-10 ml is usually sufficient. Specimens should be taken at the most appropriate times; samples should be collected just before a dose is due and/or around the expected time of peak blood concentrations.

Transport of specimens

Unlike specimens from cases of suspected dermatophytosis which can often be stored for weeks or even months before processing, specimens for mycological investigation must be processed as soon as possible after collection. Delay may result in the death of fastidious organisms, in overgrowth of contaminants, and/or multiplication in the number of organisms present.

Specimens mailed to laboratories must be packaged and labelled according to the guidelines laid down for the transport of biological material by the relevant postal authorities. Metal canisters are now recommended for packaging of certain hazardous materials such as specimens from HIV-infected persons. Plastic petri dishes are unsuitable for sending through mail. The specimen container or culture should be sealed within a plastic bag before packaging so that any breakage and subsequent spillage is contained. The sender's name should be clearly marked on the outside of the package so that they may be contacted for instructions should a problem arise.

Interpretation of laboratory test results

Interpretation of the results of laboratory tests can sometimes be made with confidence, but at times the findings may be unhelpful or even misleading. The investigations available include microscopic examination, culture and serological tests. The choice of appropriate tests differs from one disease to another and depends on the site of infection as well as the presenting symptoms and clinical signs. It must always be appreciated that every laboratory test has its limitations, and that negative results can be obtained which may lead to unjustified exclusion of a mycological diagnosis.

Direct microscopic examination

The direct microscopic examination of clinical material is one of the simpler and most helpful procedures for the laboratory diagnosis of fungal infection. Various methods can be used: unstained wet-mount preparations may be examined by light-field, dark-field or phase-contrast illumination; or dried smears can be stained and examined. Chemical brighteners, such as calcofluor white, can be helpful in revealing fungal elements in wet mounts of sputum, skin and other clinical materials when examined under a fluorescence microscope.

Direct microscopic examination is most useful in the diagnosis of superficial and subcutaneous fungal infections. Recognition of fungal elements in skin scrapings, hair or nail specimens can provide a reliable indication of the mycosis involved, whether it be dermatophytosis, candidosis or pityriasis versicolor. In certain situations, direct microscopic examination of fluids or other clinical material can establish the diagnosis of a deep mycosis. Instances include the detection of encapsulated C. ***neoformans*** cells in CSF, or ***Histoplasma capsulatum*** cells in peripheral blood smears. More often, however, only a tentative diagnosis of deep fungal infection can be made on the basis of microscopic examination.

Nevertheless, microscopic examination can help to determine whether an organism recovered later in culture is a contaminant or a pathogen, and to assist the laboratory in selecting the most appropriate culture conditions to recover organisms visualized on direct smear.

Histopathological examination

Histopathological examination of tissue sections is one of the most reliable procedures for the diagnosis of subcutaneous and deep-seated fungal infections. However, the ease with which a fungal pathogen can be recognized in tissue is dependent not only on its abundance but also on the distinctiveness of its appearance. Many fungi stain poorly with hematoxylin and eosin and this method alone may be insufficient to reveal fungal elements in tissue. There are a number of special stains for detecting and highlighting fungi and the clinician should request these if a mycotic disease is suspected.

Methenamine-silver (Grocott or Gomori) and periodic acid-Schiff staining are among the most widely used procedures for specific staining of the fungal cell wall.

It should be appreciated that these staining methods, although useful at revealing the presence of fungal elements in tissue, seldom permit the precise fungal genus involved to be identified. For example, the detection of non-pigmented branching, septate hyphae is typical of ***Aspergillus*** infection, but it is also characteristic of a large number of less common organisms, including species of ***Fusarium*** and ***Scedosporium.*** Likewise, the detection of small, budding yeast cells seldom permits a specific diagnosis. Tissue-form cells of H. ***capsulatum*** and ***Blastomyces dermatitidis,*** for instance, can appear similar and may be confused with non-encapsulated cells of C. ***neoformans.***

To overcome this problem, a number of methods have been developed for identifying various fungi in tissue. Immunoperoxidase and immunofluorescent staining reagents, both monoclonal and polyclonal, are available for some fungi. Immunochemical staining can facilitate the identification of atypical fungal elements and the detection of small numbers of organisms. It can also assist with the diagnosis of mixed infections. Currently under investigation are a number of techniques that involve specific binding of **DNA** probes to the nucleic acid of the fungal agent either directly on the slide ***(in situ*** hybridization) or in a test tube.

Culture

Isolation in culture will permit most pathogenic fungi to be identified. Most of these organisms are not fastidious in their nutritional requirements and will grow on the media used for bacterial isolation from clinical material. However, growth on these media can be slow and development of the spores and other structures used in fungal identification can be poor. For these reasons, most laboratories use several different culture media and incubation conditions for recovery of fungal agents.

Most laboratories use a medium, such as Sabouraud Dextrose Agar or Malt Agar, that will recover most common fungi. However, certain fastidious organisms such as yeast-phase H. ***capsulatum*** will not grow on these substrates, and require the use of richer media such as brain-heart infusion agar. The laboratory should be made aware of the particular fungal agent(s) that are suspected in a given sample so that the most appropriate media can be included.

Many clinical specimens submitted for fungal culture are contaminated with bacteria and it is essential to add antibacterial antibiotics to fungal culture media. Media containing chloramphenicol are commercially available. However, gentamicin, vancomycin and other antimicrobial agents are increasingly being used to suppress growth of bacteria resistant to older agents. If dermatophytes or dimorphic fungi are being isolated, cycloheximide (actidione) should be added to the medium to prevent overgrowth by faster-growing fungi.

The optimum growth temperature for most pathogenic fungi is around 30°C. Material from patients with a suspected superficial infection should be incubated at 25-30°C, because most dermatophytes will not grow at higher temperatures. Material from subcutane**ous** or deep sites should be incubated at two temperatures, 25-30°C and 37°C. This is because a number of important pathogens, including H. ***capsulatum, B. dermatitidis*** and ***Sporothrix schenckii,*** are dimorphic and the change in their growth form, depending on the incubation conditions, is useful in identification. At 25-30°C these organisms develop as moulds on glucose peptone agar, but at higher temperatures on an enriched medium, such as brain-heart infusion agar, these organisms grow as budding yeasts. Some pathogenic fungi grow slowly in culture and require plates to be held for up to 2 weeks, and in some case up to **4** weeks, before being discarded as negative. However, many common pathogenic fungi, such as ***Aspergillus fumigates*** and ***Candida albicans,*** will produce identifiable colonies within 1-3 days. Cultures should be examined at frequent intervals (at least three times weekly) and appropriate subcultures made, particularly from blood enriched media on which fungi often fail to sporulate.

It is important to recognize that growth of an organism in culture does not necessarily establish its role as a pathogen. Only if the

organism is identified as an unequivocal pathogen, such as ***Trichophyton rubrum*** or H. ***capsulatum,*** can the diagnosis be firmly established. If, however, an opportunistic organism such as
A. fumigatus or ***C. albicans*** is recovered, its isolation may have no clinical relevance unless there is additional evidence of its involvement in a pathogenic process. In this situation, culture results should be compared with those of microscopic examination. Isolation of opportunistic fungal pathogens from sterile sites, such as blood or CSF, often provides reliable evidence of significant infection, but their isolation from material such as pus, sputum or urine must be interpreted with caution.
Although culture often provides the definitive diagnosis of a fungal infection, it also has some limitations.
Chief amongst these is failure to recover the organism. This may be due to inadequate specimen collection or delayed transport of specimens. Incorrect isolation procedures or inadequate periods of incubation are other important factors. It is essential for the clinician to inform the laboratory if a particular fungal infection is suspected and provide sufficient information to permit the most appropriate culture procedures to be followed.
The isolation and identification of moulds and yeasts can take several weeks. In such unavoidable instances, the result may become available too late either to help with the diagnosis or with the choice of treatment. Nevertheless, culture should always be attempted so that a definitive diagnosis can be obtained.

Serological tests

Serological testing often provides the most rapid means of diagnosing a fungal infection. The majority of tests are based on the detection of antibodies to specific fungal pathogens, although tests for fungal antigens are now becoming more widely available. At their best, individual serological tests can be diagnostic, for example, tests for antigenaemia in cryptococcosis and histoplasmosis.
In general, however, the results of serological testing are seldom more than suggestive or supportive of a fungal diagnosis. These tests must be interpreted with caution and considered alongside the results of other clinical and laboratory investigations.
Tests for antibodies have proved useful in diagnosing endemic fungal diseases, such as histoplasmosis and coccidioidomycosis, in immunocompetent persons. In these individuals, the interval between exposure and the development of symptoms **(2-6** weeks) is usually sufficient for a humoral response to develop. Tests for fungal antibodies are most helpful when paired serum specimens (acute and convalescent) are obtained, ***so*** that it can be determined whether titres are rising or falling. Tests for detection of antibodies are much less useful in immunocompromised persons, many of whom are incapable of mounting a detectable humoral response to infection.
In this situation, serological tests for detecting fungal antigens can be helpful. Antigen detection is an established procedure for the diagnosis of cryptococcosis and histoplasmosis, and similar tests are currently being evaluated for aspergillosis and candidosis. Antigen detection methods are complicated by several important factors. First, antigen is often released in minute amounts from fungal cells necessitating the use of highly sensitive test procedures to detect low amounts circulating in serum. Second, antigen is often cleared very rapidly from the circulation necessitating frequent collection of samples. Third, antigen is often bound to circulating IgG, even in immunocompromised individuals, and therefore steps must be taken to dissociate these complexes before antigen can be detected.
Numerous tests are available for the detection of fungal antibodies. Immunodiffusion (ID) is a simple, specific and inexpensive method, but it is insensitive and this reduces its usefulness as a screening test. Complement fixation (CF) is more sensitive, but more difficult to perform and interpret than ID. However, CF remains an important test for a number of fungal diseases, including histoplasmosis and coccidioidomycosis. Latex agglutination is a simple but insensitive method that can be used for detection of antibodies or antigens. It has proved most useful for detection of the polysaccharide capsular antigens of C. ***neoformans*** that are released in large amounts in most patients with cryptococcosis.
More sensitive procedures, such as radioimmunoassay and enzyme-linked immunosorbent assay (ELISA), have also been developed and evaluated for the diagnosis of a number of fungal diseases.

Molecular diagnosis of fungal infection

Numerous methods have been developed and evaluated for the detection of fungal nucleic acid sequences in blood, CSF, respiratory tract fluids and other clinical samples. Most of these molecular diagnostic tests are based on the use of the polymerase chain reaction (PCR) to take advantage of the enormous increase in sensitivity offered by the manifold amplification of PCR targets as well as the specificity offered by appropriate primer and probe design. Several regions within the fungal genome have been evaluated as potential targets for detection, but much effort has focused on the ribosomal DNA (rDNA) gene complex. This section of the genome includes the relatively conserved regions of the ***18S, 5.8s*** and *28s* genes and the more variable intervening transcribed spacer (ITS) regions.
Despite recent progress, the goal of developing rapid and cost-effective tests for the molecular diagnosis of acute life-threatening fungal infections remains elusive. Although numerous research laboratories now offer 'in-house' procedures for molecular detection of fungal infection from tissue specimens or from body fluids, the sensitivity, specificity and predictive value of these tests have not always been thoroughly investigated. It is to be hoped that, in the future, the relevance of these assays will be demonstrated and that they will become available to a much broader group of clinical laboratories.

Antonie van Leeuwenhoek

Born October 24, 1632, Delft, Dutch Republic
Died August 26, 1723 (aged 90), Delft, Dutch Republic
Residence Netherlands
Nationality Dutch
Fields Microscopist and Biologist

Antonie van Leeuwenhoek is the somewhat improbable father of microbiology. A moderately educated owner of a textile business, he learned how to make his own unique microscopes which offered unparallelled magnification. Using these microscopes he made a number of crucially important scientific discoveries, including single-celled animals and plants, bacteria, and spermatozoa.

His microscopy methods were so finely tuned that after he discovered bacteria, this type of organism would not be observed again by any other scientist for over 100 years.

Beginnings

Antonie van Leeuwenhoek was born on October 24, 1632, in the small city of Deflt in the Dutch Republic. His father was Philips Antonisz van Leeuwenhoek, a basket maker. His mother was Margaretha Bel van den Berch, whose prosperous family were beer brewers.

Antonie's early life was rather rocky: his father died when he was just five years old. His mother remarried, and Antonie spent some time living with an uncle. His uncle was a lawyer and helped Antonie with basic literacy and numeracy, reinforcing the education he had received in local schools. By the time Antonie was 16, his step-father had also died.

Antonie learned no languages other than Dutch, which suggests he was never expected to go to university: he would have needed to learn at least Latin for this.

Business Career

In 1648, at the age of 16, Leeuwenhoek moved to the famous Dutch trading city of Amsterdam to begin work in a textile shop. He learned his trade well and was promoted to the trusted position of cashier and book-keeper.

In 1654, aged 21, he returned to Delft, where he would spend the rest of his long life. This was a significant year for Leeuwenhoek. Not only did he return to his hometown, but he got married, and, putting his business experience in Amsterdam into practice, he opened his own textile shop in Delft. In addition to cloth he sold buttons, ribbons, and other accessories.

Over the next few years Leeuwenhoek became an influential figure in Delft. In 1660, aged 28, he was appointed to manage the operations of Delft's council meeting hall. In return for only a small amount of work – the actual physical work of the job was delegated to other people – he received a generous salary.

A man of many talents, Leeuwenhoek was also appointed to supervise Delft's wine trade and levy the appropriate taxes on imported wine.

While running his shop and working for the city of Delft, Leeuwenhoek became a qualified land surveyor at about 40 years of age, just before he started his scientific work.

The Discovery of the Leeuwenhoek Lens

Leeuwenhoek's biological discoveries were completely dependent on his ability to make lenses of extraordinarily high quality.

He never told anyone how he made his lenses. The secret went with him to the grave. In fact, to throw competitors off the scent, he used to talk about how he had to grind glass for a very long time to make his lenses. This was almost certainly not true.

Glass Pearls

People in the textiles trade had, for hundreds of years, used glass pearls – small spheres of glass – as lenses to examine cloth in fine detail. Leeuwenhoek would have used glass pearls frequently in his day-to-day business to examine the density of threads and the quality of cloth.

Micrographia

In 1665 the great English scientist Robert Hooke released *Micrographia* showcasing drawings he had made of the natural world seen through the lens of his microscope.

Leeuwenhoek visited England in 1668 and most likely saw a copy of *Micrographia*: it had become the first scientific bestseller. Importantly for Leeuwenhoek, it contained drawings Hooke had made of his microscopic examinations of cloth.

We cannot tell whether Leeuwenhoek was aware of Hooke's words – he could not read English. However, it is now believed that he used Hooke's technique to make his lenses.

Hooke himself did not use lenses made by this method, because they were inconvenient: the distance between the lens and the viewed object had to be very short and the observer's eye had to be pushed very close to the lens, causing Hooke's eyes to quickly become strained.

Hooke used a compound microscope (one with two lenses) which more closely resembles the microscopes we use today.

Lens Making

Leeuwenhoek, however, was more than happy to use small, spherical lenses to make single-lens microscopes. He kept the details of how he manufactured his lenses secret, but today we can be reasonably sure that he did the following:

- used a hot flame to heat the middle part of a glass rod until molten
- pulled the ends of the rod in opposite directions, forming a long, thin thread of molten glass
- continued pulling the ends while the molten thread in the middle grew thinner and thinner, finally breaking
- placed one of the thread ends back into the flame, resulting in the end of the thread forming a small glass sphere

This sphere was a lens, which may have required some polishing. The smaller the sphere, the greater the magnification.

When he started making lenses, Leeuwenhoek may have hoped to use them to examine textiles more closely than anyone had ever done before.

Soon, however, he felt the same compulsion as Hooke had felt to examine natural objects in never-before-seen detail.

Leeuwenhoek's Microscopes

Microscopes made from Leeuwenhoek's tiny spherical lenses – the smallest lenses measured just 1 mm across – were easily capable of magnifying objects by a factor of about 200 – 300, while Hooke's compound microscope magnified only by a factor of about 40 – 50.

Remarkably, Leeuwenhoek could use his lenses to resolve details

as small as 1.35 µm. (This meant that, for example, he could easily see red blood cells, which are typically 6 – 8 µm in diameter.)

Leeuwenhoek's Art of Microscopy

What is still uncertain even today is how Leeuwenhoek lit the objects he was studying. This was a vitally important part of his unique art of microscopy. Another was his unrivaled skill in setting up for viewing:

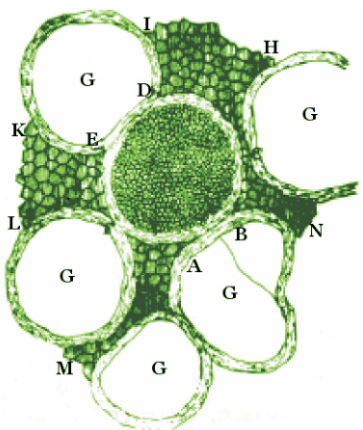
- drops of liquid, such as blood or pond-water, or
- solid samples, such as plant material or animal muscles, cleanly cut with a razor blade into very thin sections, transparent enough for light to travel through so their details could be seen and drawn.

Leeuwenhoek made over 500 tiny microscopes during his lifetime. They were awkward to use and inconvenient, which is why today we now use compound microscopes. Despite their drawbacks, in Leeuwenhoek's expert hands, they revealed an entirely new biological world.

The Microscopic World Discovered by Leeuwenhoek

Leeuwenhoek was a tradesman who had no formal training in science and had never been to university.

Nevertheless, the quality of his observations was so high and his discoveries so compelling that his research became well-known through letters he sent to the Royal Society in London. These were translated into English and published in the Society's journal, *Philosophical Transactions*.



A cross-sectional view of a nerve fiber drawn by Antonie van Leeuwenhoek.

Interestingly, many of Leeuwenhoek's letters were read first by Robert Hooke, who was the Curator of Experiments and then Secretary of the Society. Hooke actually learned Dutch so he could read Leeuwenhoek's letters for himself.

Leeuwenhoek's first communication was in 1673, replicating some of the work Hooke had covered in *Micrographia*, including Leeuwenhoek's detailed drawings of bee stings, a fungus, and a human louse.

The following year Leeuwenhoek began to describe outstanding new discoveries he had made.

Discoveries

Single-Celled Life

In 1674, aged 41, Leeuwenhoek made the first of his great discoveries: single-celled life forms. Nowadays these organisms are grouped with the *protists* – these are mainly single-celled plants and animals. Echoing the initial disbelief Hooke's *Micrographia* had met, many members of the Royal Society refused to believe in the existence of Leeuwenhoek's microscopic creatures. It took until 1677 before their existence was fully accepted. This happened after Robert Hooke returned to his microscopes, which he had given up because of eye strain, and verified Leeuwenhoek's observations.

The Shape and Size of Red Blood Cells

In 1674 Leeuwenhoek examined red blood cells, which had been discovered six years earlier by his fellow Dutchman, Jan Swammerdam. With his superior lens, Leeuwenhoek was able to give a clearer description of the cells than ever before and was the first person to accurately determine their size.

Bacteria

In 1676 Leeuwenhoek discovered bacteria in water. The bacteria were at the limit of observation of his microscope – he estimated that it would take more than 10,000 of them to fill the volume of a small grain of sand. Such was the brilliance of his work that nobody else observed bacteria until another century had passed.

Spermatozoa

In 1677 Leeuwenhoek discovered spermatozoa, later concluding that eggs are fertilized when entered by sperm.

Lymphatic Capillaries

In 1683 Leeuwenhoek discovered the lymphatic capillaries, which contained "a white fluid, like milk."

Even More Discoveries

By observing the life-cycles of maggots and fleas Leeuwenhoek proved that such creatures are not spontaneously generated, as many people believed at the time. He showed these creatures go through a process of reproduction from eggs to maggots to pupae to adults.

By dissecting aphids he discovered parthenogenesis. He found parent aphids containing the embryos of new aphids although eggs had not been fertilized.

By observing the flow of blood in tiny capillaries, Leeuwenhoek confirmed William Harvey's work on blood circulation.

The Birth of a New Science

Leeuwenhoek's discoveries, combined with Hooke's earlier discovery of microscopic fungi, signaled the creation of a new science: microbiology.

Some Personal Details and the End

Leeuwenhoek married Barbara de Mey in 1654, when he was 21 years old. They had five children, but only one – their daughter Maria – survived beyond infancy.

Barbara died in 1666, after twelve years of marriage. Five years later Leeuwenhoek married Cornelia Swalmius, with whom he had no children. It was during his second marriage that Leeuwenhoek's interest in science seems to have developed.

In February 1680 Leeuwenhoek was elected to The Royal Society in London. He took great pride in this; it meant that he had won recognition as a true scientist. He never visited the Royal Society. He was happy to continue his work in Delft.

Leeuwenhoek's second wife, Cornelia, died in 1694, when Leeuwenhoek was 61 years old.

Antonie van Leeuwenhoek died aged 90 on August 26, 1723. He was buried in the Old Church in Delft.

Although he had not been born into a scientific family nor had he received an education in science, his death was that of a true scientist. He communicated to the Royal Society such a careful, detailed description of the medical condition that was affecting him, eventually causing his death, that it is now called Van Leeuwenhoek's disease. It is a rare condition which causes involuntary twitching of muscles.

Leeuwenhoek was survived by his daughter Maria, who had not married. She looked after her aging father and helped him to run the family's textile business. Leeuwenhoek had become a wealthy man and Maria inherited this wealth.

JOKES

A man lost on no-man's-land Island.
1 day he decided to build a wood boat to save his life.
Suddenly a höt girl came there & the man use the wood 4 making bed.
Moral- A girl can change ur göal.

Dog was Chasing Titu
Titu runs, but Laughing..
A Man asked y r u Laughing? Titu replied
I hav put Vodaphone Sim, but the Hutch network is Folowing...

After robbing the bank, 1 robber to clerk : Did you see me robbing?
Clerk : Yes I saw u.
Robber killed him and asked to the next clerk : Did u?
Second Clerk : No, but my wife saw u!

Titu STD booth pe gaye or STD wale ke 2 thappad lagaye.
STD wale ne pucha, "Kyun mara".
Titu bola,"Samne sign board pe likha hai "2" laga ke dial kare."

Santa got into a bus on 1st April when conductor asked for ticket. He gave Rs.10/- and took the ticket and said April fool. I have pass.

An astronomer was watching the sky from his telescope - Santa Singh was observing him,
Suddenly a star falls, seeing that Santa Singh shouted, "Kya nishana lagaya hai!" waah....waah...

Santa by mistake goes into a ladies toilet.
All ladies suddenly stand up
Santa : Izzat dil me ho yehi kaafi hai,
Baitho Baitho…:)

Madam to Student : Last Semester you were roaming with that girl and this semester you are roaming with other.
What you think of yourself?
Boy: Syllabus changed mam.

QUIZ

1) A disadvantage of gas sterilization is
a) it raises the temperature in the unit to 270 degrees F.
b) it uses ethylene oxide which is hazardous to humans and to the environment.
c) the instruments must be placed in a small chamber.
d) it generally takes up to 10 hours to complete the process.

2) Which of the following is commonly used in the laboratory setting to provide a measure of protection against transmission of the human immunodeficiency virus (HIV)?
a) germicidal soap c) iodine
b) boiling water d) bleach

3) Which of the following methods of infection control requires the use of an autoclave?
a) disinfection c) sterilization
b) ultrasonic cleaning d) sanitization

4) Which of the following is used to disinfect surfaces and soak rubber equipment before sanitization?
a) bleach b) alcohol c) iodine

5) Which of the following is permissible in ultrasonic cleaning?
a) place all instruments together in the special bath
b) place all instruments with hinges or ratchets in the ultrasonic cleaner in the closed position
c) remove the instruments from the ultrasonic cleaner and immediately wrap in a towel without rinsing

6) Which of the following is used to clean delicate instruments or instruments with moving parts?
a) sterilization c) sanitization
b) disinfection d) ultrasonic cleaning

7) Which of the following is used both as a disinfectant and antiseptic but its effectiveness is limited by the presence of blood products, mucus, or soap?
a) boiling water b) iodine c) acid products

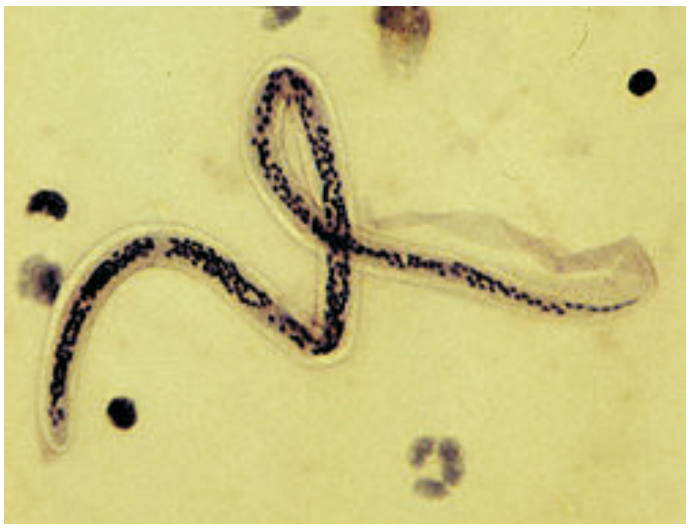
8) Which factor has an impact on the effectiveness of a disinfectant?
a) the disinfectant solution is more potent after several uses
b) items should be placed in the disinfectant bath wet
c) using soap in the sanitization process is desirable
d) evaporation can alter the chemical makeup of the solution

9) `The cleaning process that is required for all instruments that penetrate the skin or that come in contact with normally sterile areas of the tissue and internal organs is
a) sterilization. c) disinfection.
b) sanitization. d) ultrasonic cleaning.

10) Which of the following can be disinfected and reused without sterilization?
a) needles and syringes c) vaginal specula
b) glassware and enamelware d) curettes

Answers : 1)B 2)D 3)C 4)A 5)D 6)D 7)B 8)D 9)A 10)B

Wuchereria bancrofti



Kingdom:	Animalia	Phylum:	Nematoda
Class:	Secernentea	Order:	Spirurida
Suborder:	Spirurina	Family:	Onchocercidae
Genus:	*Wuchereria*	Species:	*W. bancrofti*

Wuchereria bancrofti is a human parasitic roundworm that is the major cause of lymphatic filariasis. It is one of the three parasitic worms, besides *Brugia malayi* and *B. timori*, that infect lymphatic system to cause lymphatic filariasis. These filarial worms are spread by a mosquito vector. *W. bancrofti* is the most prevalent among the three and affects over 120 million people, primarily in Central Africa and the Nile delta, South and Central America, and the tropical regions of Asia including southern China and the Pacific. If the infection is left untreated, it can develop into a chronic disease called elephantiasis. In rare conditions it also causes tropical eosinophilia, an asthmatic disease. Limited treatment modalities exist and no vaccines have been developed.

History

The effects of *W. bancrofti* were documented early in ancient text. Ancient Greek and Roman writers noted the similarities between the enlarged limbs and cracked skin of infected individuals to that of elephants. Since then, this condition has been commonly known as elephantiasis. However, this is a misnomer, since elephantiasis literally translates to "a disease caused by elephants".

W. bancrofti was named after physician Otto Wucherer and parasitologist Joseph Bancroft, both of whom extensively studied filarial infections.

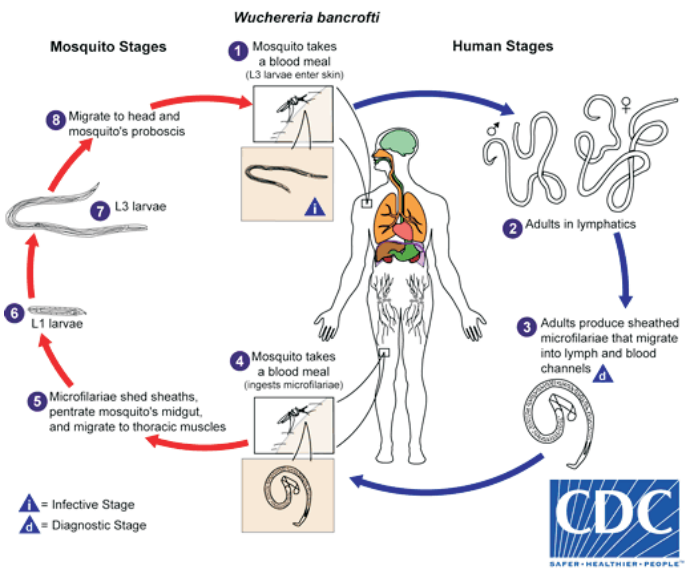
W. bancrofti is speculated to have been brought to the New World by the slave trade. Once it was introduced to the New World, this filarial worm disease persisted throughout the areas surrounding Charleston, South Carolina until its sudden disappearance in the 1920s.

Morphology

As dioecious worm, *W. bancrofti* exhibits sexual dimorphism. The adult worm is long, cylindrical, slender, and smooth with rounded ends. It is white in colour and almost transparent. The body is quite delicate making it difficult to remove from tissues. It has a short cephalic or head region connected to the main body by a short neck which appears as a constriction. There are dark spots which are dispersed nuclei throughout the body cavity, with no nuclei at the tail tip. Male and female can be differentiated by size and structure of tail tip. The male worm is smaller, 40 millimetres (1.6 in) long and 100 micrometres (0.0039 in) wide, and features a ventrally curved tail. The tip of the tail has 15 pairs of minute caudal papillae, the sensory organs. The anal region is an elaborate structure consisting of 12 pairs of papillae, of which 8 are in front and 4 are behind the anus. In contrast, the female is 60 millimetres (2.4 in) to 100 millimetres (3.9 in) long and 300 micrometres (0.012 in) wide, nearly three times larger in diameter than the male. Its tail gradually tapers and rounded at the tip. There are no additional sensory structures. Its vulva lies towards the anterior region, about 0.25 mm from the head. Adult male and female are most often coiled together and are difficult to separate. Females are ovoviviparous and can produce thousands of juveniles known as microfilariae.

The microfilaria is a miniature adult and retains the egg membrane as a sheath and are often considered advanced embryos. It measures 280 µm long and 25 µm wide. It is quite structureless in living condition. However by histological staining it is shown to have primitive gut, nerve ring and muscles.

Life Cycle of *Wuchereria bancrofti*



Different species of the following genera of mosquitoes are vectors of *W. bancrofti* filariasis depending on geographical distribution. Among them are: *Culex* (*C. annulirostris, C. bitaeniorhynchus, C. quinquefasciatus,* and *C. pipiens*); *Anopheles* (*A. arabinensis, A. bancroftii, A. farauti, A. funestus, A. gambiae, A. koliensis, A. melas, A. merus, A. punctulatus* and *A. wellcomei*); *Aedes* (*A. aegypti, A. aquasalis, A. bellator, A. cooki, A. darlingi, A. kochi, A. polynesiensis, A. pseudoscutellaris, A. rotumae,A. scapularis*, and *A. vigilax*); *Mansonia* (*M. pseudotitillans, M. uniformis*); *Coquillettidia* (*C. juxtamansonia*). During a blood meal, an infected mosquito introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound 1. They develop in adults that commonly reside in the

lymphatics 2. The female worms measure 80 to 100 mm in length and 0.24 to 0.30 mm in diameter, while the males measure about 40 mm by .1 mm. Adults produce microfilariae measuring 244 to 296 µm by 7.5 to 10 µm, which are sheathed and have nocturnal periodicity, except the South Pacific microfilariae which have the absence of marked periodicity. The microfilariae migrate into lymph and blood channels moving actively through lymph and blood 3. A mosquito ingests the microfilariae during a blood meal 4. After ingestion, the microfilariae lose their sheaths and some of them work their way through the wall of the proventriculus and cardiac portion of the mosquito's midgut and reach the thoracic muscles 5. There the microfilariae develop into first-stage larvae 6 and subsequently into third-stage infective larvae 7. The third-stage infective larvae migrate through the hemocoel to the mosquito's prosbocis 8 and can infect another human when the mosquito takes a blood meal

Epidemiology & Risk Factors

There are three different filarial species that can cause lymphatic filariasis in humans. Most of the infections worldwide are caused by *Wuchereria bancrofti*. In Asia, the disease can also be caused by *Brugia malayi* and *Brugia timori*.

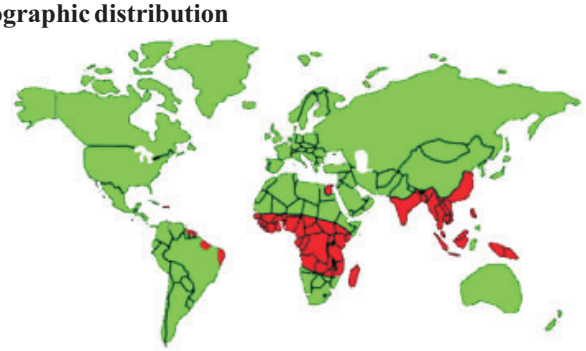
The infection spreads from person to person by mosquito bites. The adult worm lives in the human lymph vessels, mates, and produces millions of microscopic worms, also known as microfilariae. Microfilariae circulate in the person's blood and infect the mosquito when it bites a person who is infected. Microfilariae grow and develop in the mosquito. When the mosquito bites another person, the larval worms pass from the mosquito into the human skin, and travel to the lymph vessels. They grow into adult worms, a process that takes 6 months or more. An adult worm lives for about 5–7 years. The adult worms mate and release millions of microfilariae into the blood. People with microfilariae in their blood can serve as a source of infection to others.

A wide range of mosquitoes can transmit the parasite, depending on the geographic area. In Africa, the most common vector is Anopheles and in the Americas, it is *Culex quinquefasciatus*. *Aedes* and *Mansonia* can transmit the infection in the Pacific and in Asia.

Many mosquito bites over several months to years are needed to get lymphatic filariasis. People living for a long time in tropical or sub-tropical areas where the disease is common are at the greatest risk for infection. Short-term tourists have a very low risk.

Programs to eliminate lymphatic filariasis are under way in more than 50 countries. These programs are reducing transmission of the filarial parasites and decreasing the risk of infection for people living in or visiting these communities.

Geographic distribution



The areas in red indicate the geographic distribution of lymphatic filariasis.

Lymphatic filariasis affects over 120 million people in 73 countries throughout the tropics and sub-tropics of Asia, Africa, the Western Pacific, and parts of the Caribbean and South America.

In the Americas, only four countries are currently known to be endemic: Haiti, the Dominican Republic, Guyana and Brazil.

In the United States, Charleston, South Carolina, was the last known place with lymphatic filariasis. The infection disappeared early in the 20th century. Currently, you cannot get infected in the U.S.

Diagnosis

A blood smear is a simple and fairly accurate diagnostic tool, provided the blood sample is taken during the period in the day when the juveniles are in the peripheral circulation. Technicians analyzing the blood smear must be able to distinguish between *W. bancrofti* and other parasites potentially present.

A polymerase chain reaction test can also be performed to detect a minute fraction, as little as 1 pg, of filarial DNA. Some infected people do not have microfilariae in their blood. As a result, tests aimed to detect antigens from adult worms can be used.

Ultrasonography can also be used to detect the movements and noises caused by the movement of adult worms. Dead, calcified worms can be detected by X-ray examinations.

Treatment

The severe symptoms caused by the parasite can be avoided by cleansing the skin, surgery, or the use of therapeutic drugs, such as diethylcarbamazine (DEC), ivermectin, oralbendazole. The drug of choice, however, is DEC, which can eliminate the microfilariae from the blood and also kill the adult worms with a dosage of 6 mg/kg semiannually or annually. A polytherapy treatment that includes ivermectin with DEC or albendazole is more effective than each drug alone. Protection is similar to that of other mosquito-spread illnesses; one can use barriers both physical (a mosquito net), chemical (insect repellent), or mass chemotherapy as a method to control the spread of the disease.

Mass chemotherapy should cover the entire endemic area at the same time. This will significantly decrease the overall microfilarial titer in blood in mass, hence decreasing the transmission through mosquitoes during their subsequent bites.

Antibiotic active against the Wolbachia symbionts of the worm have been experimented with as treatment.

Control

Prevention focuses on protecting against mosquito bites in endemic regions. Insect repellents and mosquito nets are useful to protect against mosquito bites. Public education efforts must also be made within the endemic areas of the world to successfully lower the prevalence of *W. bancrofti* infections.

Eradication

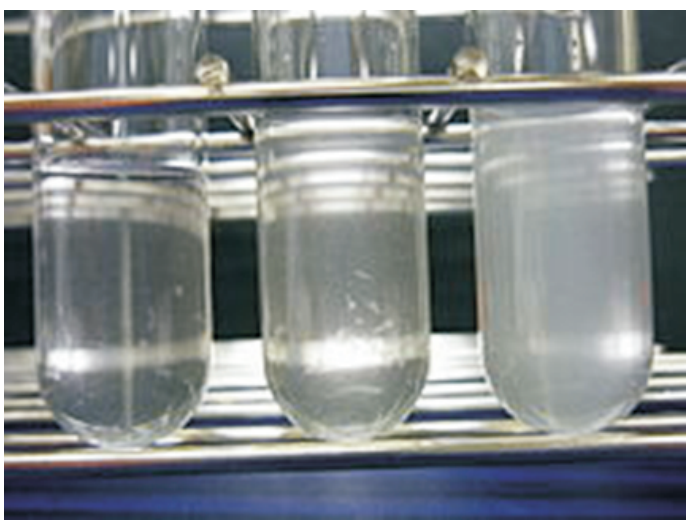
The WHO is coordinating an effort to eradicate filarisis. The mainstay of this programme is the mass use of antifilarial drugs on a regular basis for at least five years.

In April 2011, Sri Lanka was certified by the WHO as having eradicated this disease.

McFarland standards

In microbiology, McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing.

An example of such testing is antibiotic susceptibility testing by measurement of minimum inhibitory concentration which is routinely used in medical microbiology and research. If a suspension used is too heavy or too dilute, an erroneous result (either falsely resistant or falsely susceptible) for any given anti microbial agent could occur.



Original McFarland standards were mixing specified amounts of barium chloride and sulfuric acid together. Mixing the two compounds forms a barium sulfate precipitate, which causes turbidity in the solution. A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4).

Now there are McFarland standards prepared from suspensions of latex particles, which lengthens the shelf life and stability of the suspensions. The standard can be compared visually to a suspension of bacteria in sterile saline or nutrient broth. If the bacterial suspension is too turbid, it can be diluted with more diluent. If the suspension is not turbid enough, more bacteria can be added.

McFarland Nephelometer Standards:

McFarland Standard No.	0.5	1	2	3	4
1.0% Barium chloride (ml)	0.05	0.1	0.2	0.3	0.4
1.0% Sulfuric acid (ml)	9.95	9.9	9.8	9.7	9.6
Approx. cell density (1X10^8 CFU/mL)	1.5	3.0	6.0	9.0	12.0
% Transmittance*	74.3	55.6	35.6	26.4	21.5
Absorbance*	0.08 to 0.1	0.257	0.451	0.582	0.669

*at wavelength of 600 nm

McFarland Latex Standards from Hardy Diagnostics (2014-12-10), measured at the UCSF DeRisi Lab

McFarland Standard No.	0.5	1	2	4	6	8
Absorbance @600nm	0.063	0.123	0.242	0.431	0.653	0.867

Microbiology lab: Health & Safety-Part 2

Safety checklist

This checklist is intended to assist in assessments of microbiological laboratory safety and security status of biomedical laboratories.

Laboratory premises

1. Have guidelines for commissioning and certification been considered for facility construction or post-construction evaluations?
2. Do the premises meet national and local building requirements, including those relating to natural disaster precautions if necessary?
3. Are the premises generally uncluttered and free from obstructions?
4. Are the premises clean?
5. Are there any structural defects in floors?
6. Are floors and stairs uniform and slip-resistant?
7. Is the working space adequate for safe operation?
8. Are the circulation spaces and corridors adequate for the movement of people and large equipment?
9. Are the benches, furniture and fittings in good condition?
10. Are bench surfaces resistant to solvents and corrosive chemicals?
11. Is there a hand-washing sink in each laboratory room?
12. Are the premises constructed and maintained to prevent entry and harbourage of rodents and arthropods?
13. Are all exposed steam and hot water pipes insulated or guarded to protect personnel?
14. Is an independent power support unit provided in case of power breakdown?
15. Can access to laboratory areas be restricted to authorized personnel?
16. Has a risk assessment been performed to ensure that appropriate equipment and facilities are available to support the work being considered?

Storage facilities

1. Are storage facilities, shelves, etc. arranged so that stores are secure against sliding, collapse or falls?
2. Are storage facilities kept free from accumulations of rubbish, unwanted materials and objects that present hazards from tripping, fire, explosion and harbourage of pests?
3. Are freezers and storage areas lockable?

Sanitation and staff facilities

1. Are the premises maintained in a clean, orderly and sanitary condition?
2. Is drinking-water available?
3. Are clean and adequate toilet (WC) and washing facilities provided separately for male and female staff?
4. Are hot and cold water, soap and towels provided?
5. Are separate changing rooms provided for male and female staff?
6. Is there accommodation (e.g. lockers) for street clothing for individual members of the staff?
7. Is there a staff room for lunch, etc.?
8. Are noise levels acceptable?
9. Is there an adequate organization for the collection and disposal of general household rubbish?

Heating and ventilation

1. Is there a comfortable working temperature?
2. Are blinds fitted to windows that are exposed to full sunlight?
3. Is the ventilation adequate, e.g. at least six changes of air per hour, especially in rooms that have mechanical ventilation?
4. Are there HEPA filters in the ventilation system?
5. Does mechanical ventilation compromise airflows in and around biological safety cabinets and fume cupboards?

Lighting

1. Is the general illumination adequate (e.g. 300–400 lx)?
2. Is task (local) lighting provided at work benches?
3. Are all areas well-lit, with no dark or ill-lit corners in rooms and corridors?
4. Are fluorescent lights parallel to the benches?
5. Are fluorescent lights colour-balanced?

Services

1. Is each laboratory room provided with enough sinks, water, electricity and gas outlets for safe working?
2. Is there an adequate inspection and maintenance programme for fuses, lights, cables, pipes, etc.?
3. Are faults corrected within a reasonable time?
4. Are internal engineering and maintenance services available, with skilled engineers and craftsmen who also have some knowledge of the nature of the work of the laboratory?
5. Is the access of engineering and maintenance personnel to various laboratory areas controlled and documented?
6. If no internal engineering and maintenance services are available, have local engineers and builders been contacted and familiarized with the equipment and work of the laboratory?
7. Are cleaning services available?
8. Is the access of cleaning personnel to various laboratory areas controlled and documented?
9. Are information technology services available and secured?

Laboratory biosecurity

1. Has a qualitative risk assessment been performed to define risks that a security system should protect against?
2. Have acceptable risks and incidence response planning parameters been defined?
3. Is the whole building securely locked when unoccupied?
4. Are doors and windows break-proof?
5. Are rooms containing hazardous materials and expensive equipment locked when unoccupied?
6. Is access to such rooms, equipment and materials appropriately controlled and documented?

Fire prevention and fire protection

1. Is there a fire alarm system?
2. Are the fire doors in good order?
3. Is the fire detection system in good working order and regularly tested?
4. Are fire alarm stations accessible?
5. Are all exits marked by proper, illuminated signs?
6. Is access to exits marked where the routes to them are not immediately visible?

7. Are all exits unobstructed by decorations, furniture and equipment, and unlocked when the building is occupied?
8. Is access to exits arranged so that it is not necessary to pass through a high-hazard area to escape?
9. Do all exits lead to an open space?
10. Are corridors, aisles and circulation areas clear and unobstructed for movement of staff and fire-fighting equipment?
11. Is all fire-fighting equipment and apparatus easily identified by an appropriate colour code?
12. Are portable fire extinguishers maintained fully charged and in working order, and kept in designated places at all times?
13. Are laboratory rooms with potential fire hazards equipped with appropriate extinguishers and/or fire blankets for emergency use?
14. If flammable liquids and gases are used in any room, is the mechanical ventilation sufficient to remove vapours before they reach a hazardous concentration?
15. Are personnel trained to respond to fire emergencies?

Flammable liquid storage

1. Is the storage facility for bulk flammable liquids separated from the main building?
2. Is it clearly labelled as a fire-risk area?
3. Does it have a gravity or mechanical exhaust ventilation system that is separate from the main building system?
4. Are the switches for lighting sealed or placed outside the building?
5. Are the light fittings inside sealed to protect against ignition of vapours by sparking?
6. Are flammable liquids stored in proper, ventilated containers that are made of non-combustible materials?
7. Are the contents of all containers correctly described on the labels?
8. Are appropriate fire extinguishers and/or fire blankets placed outside but near to the flammable liquid store?
9. Are "No smoking" signs clearly displayed inside and outside the flammable liquid store?
10. Are only minimum amounts of flammable substances stored in laboratory rooms?
11. Are they stored in properly constructed flammable storage cabinets?
12. Are these cabinets adequately labelled with "Flammable liquid – Fire hazard" signs?
13. Are personnel trained to properly use and transport flammable liquids?

Compressed and liquefied gases

1. Is each portable gas container legibly marked with its contents and correctly colour coded?
2. Are compressed-gas cylinders and their high-pressure and reduction valves regularly inspected?
3. Are reduction valves regularly maintained?
4. Is a pressure-relief device connected when a cylinder is in use?
5. Are protection caps in place when cylinders are not in use or are being transported?
6. Are all compressed gas cylinders secured so that they cannot fall, especially in the event of natural disaster?
7. Are cylinders and liquid petroleum gas tanks kept away from sources of heat?
8. Are personnel trained to properly use and transport compressed and liquefied gases?

Electrical hazards

1. Are all new electrical installations and all replacements, modifications or repairs made and maintained in accordance with a national electrical safety code?
2. Does the interior wiring have an earthed/grounded conductor (i.e. a three-wire system)?
3. Are circuit-breakers and earth-fault interrupters fitted to all laboratory circuits?
4. Do all electrical appliances have testing laboratory approval?
5. Are the flexible connecting cables of all equipment as short as practicable, in good condition, and not frayed, damaged or spliced?
6. Is each electric socket outlet used for only one appliance (no adapters to be used)?

Personal protection

1. Is protective clothing of approved design and fabric provided for all staff for normal work, e.g. gowns, coveralls, aprons, gloves?
2. Is additional protective clothing provided for work with hazardous chemicals and radioactive and carcinogenic substances, e.g. rubber aprons and gloves for chemicals and for dealing with spillages; heat-resistant gloves for unloading autoclaves and ovens?
3. Are safety glasses, goggles and shields (visors) provided?
4. Are there eye-wash stations?
5. Are there emergency showers (drench facilities)?
6. Is radiation protection in accordance with national and international standards, including provision of dosimeters?
7. Are respirators available, regularly cleaned, disinfected, inspected and stored in a clean and sanitary condition?
8. Are appropriate filters provided for the correct types of respirators, e.g. HEPA filters for microorganisms, appropriate filters for gases or particulates?
9. Are respirators fit-tested?

Health and safety of staff

1. Is there an occupational health service?
2. Are first-aid boxes provided at strategic locations?
3. Are qualified first-aiders available?
4. Are such first-aiders trained to deal with emergencies peculiar to the laboratory, e.g. contact with corrosive chemicals, accidental ingestion of poisons and infectious materials?
5. Are non-laboratory workers, e.g. domestic and clerical staff, instructed on the potential hazards of the laboratory and the material it handles?
6. Are notices prominently posted giving clear information about the location of first-aiders, telephone numbers of emergency services, etc.?
7. Are women of childbearing age warned of the consequences of work with certain microorganisms, carcinogens, mutagens and teratogens?
8. Are women of childbearing age told that if they are, or suspect that they are, pregnant they should inform the appropriate member of the medical/scientific staff so that alternative working arrangements may be made for them if necessary?
9. Is there an immunization programme relevant to the work of the laboratory?
10. Are skin tests and/or radiological facilities available for staff who work with tuberculous materials or other materials requiring such measures?
11. Are proper records maintained of illnesses and accidents?
12. Are warning and accident prevention signs used to minimize work hazards?

13. Are personnel trained to follow appropriate biosafety practices?
14. Are laboratory staff encouraged to report potential exposures?

Laboratory equipment

1. Is all equipment certified safe for use?
2. Are procedures available for decontaminating equipment prior to maintenance?
3. Are biological safety cabinets and fume cupboards regularly tested and serviced?
4. Are autoclaves and other pressure vessels regularly inspected?
5. Are centrifuge buckets and rotors regularly inspected?
6. Are HEPA filters regularly changed?
7. Are pipettes used instead of hypodermic needles?
8. Is cracked and chipped glassware always discarded and not reused?
9. Are there safe receptacles for broken glass?
10. Are plastics used instead of glass where feasible?
11. Are sharps disposal containers available and being used?

Infectious materials

1. Are specimens received in a safe condition?
2. Are records kept of incoming materials?
3. Are specimens unpacked in biological safety cabinets with care and attention to possible breakage and leakage?
4. Are gloves and other protective clothing worn for unpacking specimens?
5. Are personnel trained to ship infectious substances according to current national and/or international regulations?
6. Are work benches kept clean and tidy?
7. Are discarded infectious materials removed daily or more often and disposed of safely?
8. Are all members of the staff aware of procedures for dealing with breakage and spillage of cultures and infectious materials?
9. Is the performance of sterilizers checked by the appropriate chemical, physical and biological indicators?
10. Is there a procedure for decontaminating centrifuges regularly?
11. Are sealed buckets provided for centrifuges?
12. Are appropriate disinfectants being used? Are they used correctly?
13. Is there special training for staff who work in containment laboratories – Biosafety Level 3 and maximum containment laboratories – Biosafety Level 4?

Chemicals and radioactive substances

1. Are incompatible chemicals effectively separated when stored or handled?
2. Are all chemicals correctly labelled with names and warnings?
3. Are chemical hazard warning charts prominently displayed?
4. Are spill kits provided?
5. Are staff trained to deal with spills?
6. Are flammable substances correctly and safely stored in minimal amounts in approved cabinets?
7. Are bottle carriers provided?
8. Is a radiation protection officer or appropriate reference manual available for consultation?
9. Are staff appropriately trained to safely work with radioactive materials?
10. Are proper records of stocks and use of radioactive substances maintained?
11. Are radioactivity screens provided?
12. Are personal radiation exposures monitored?

Bibliography:

1. *Biosafety in Microbiological and Biomedical Laboratories, by* Centers for Disease Control (CDC) and the National Institutes of Health (NIH)
2. Laboratory biosafety manual: Third edition; World Health Organization, 2004

Microxpress Introduces

ULTRA PAP™

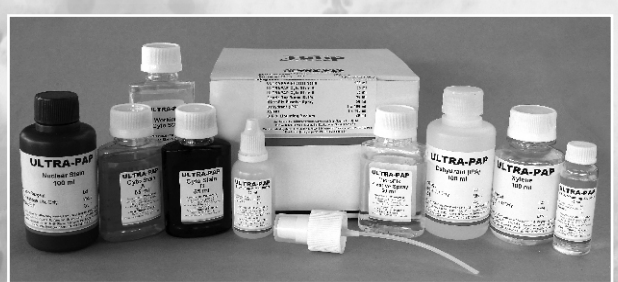
ULTRA-PAP Kit is modification of the classical PAP staining, formulated to give fast PAP staining of specimen smear with a simplified procedure thereby aiding clear nuclear and cytoplasmic staining.

Kit Contents :

ULTRAPAP – Nuclear Stain (100 ml), ULTRAPAP – Cyto-Stain A (55 ml), ULTRAPAP – Cyto-Stain B (55 ml), Scotts Tap Water Buffer (30 ml), Micro-Fix Fixative Spray (50 ml), Dehydrant (IPA) (3 x 100 ml), Xylene (2 x 100 ml), D. P. X. Mounting Medium (20 ml) and empty bottle (50ml) for preparing working cyto stain reagent.

Reagent Preparation :

As required make a Working Cyto - Stain by mixing equal amounts of ULTRAPAP Cyto - Stain A & B (An empty bottle is provided for the same). The Working Cyto - Stain is stable for at least 3 Months, provided contamination and hydration are avoided. The other contents are ready to use.



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WHO guidelines on hand hygiene in health care. ISBN 9789241597906, 2009, pg91

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FEATURES	BENEFITS
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ABS plastic	Rust free, Durable and easily cleanable
Fixed dose dispensing	Adequate disinfection Reduced wastage of handrub / handwash
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Compatible with

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TRIOSEPT™ : Colourless & odourless liquid handrub with triple action
BIOSCRUB™ : Antiseptic surgical scrub
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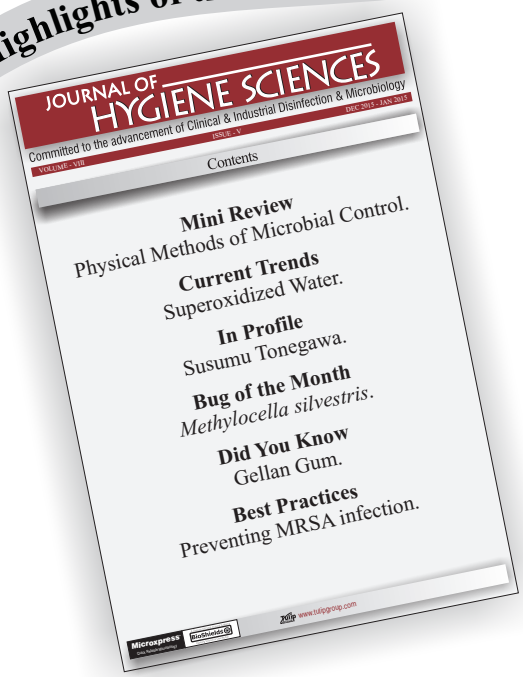
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