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Editorial

Contents

A quick glance at this issue of The Journal of Hygiene Sciences shows our continued commitment to publish a journal of high standard which is devoted exclusively to the topics of Microbiology & Disinfection.

'Mini review' section: A limited number of methods for antimicrobial susceptibility testing of medically important microorganisms have survived the maturation of modern diagnostic clinical microbiology. A variety of methods can be used to evaluate or screen the in vitro antimicrobial activity of a compound. Owing to new attraction to the properties of new antimicrobial products like combating multidrug resistant bacteria, it is important to develop a better understanding of the current methods. In this review the techniques for evaluating the in vitro antimicrobial activity are discussed in detail.

Current Trends Section: Automated Endoscope Reprocessors (AERs) are important devices widely used in the health care setting to reprocess endoscopes, such as duodenoscopes, and endoscope accessories, to decontaminate them between uses. AERs are designed to kill microorganisms in or on reusable endoscopes by exposing their outside surfaces and interior channels to chemical solutions. AERs are Class II devices cleared through the premarket notification pathway.

In Profile: Lynn Margulis, a Biologist and University professor who pioneered important concepts in the field of cell biology and microbial evolution. She is best known for her contributions towards endosymbiotic theory.

Bug of the Month: Mycoplasma Genitalium is a sexually transmitted small and pathogenic bacterium lives in the skin cells of humans urinary and genital tracts causing severe threats to humans. It is known self replicating bacterium, but its natural course of infection and importance for public health remain poorly understood.

Did you Know: Plants use sunlight to drive chemical reactions between water and CO_2 to create and store solar energy in the form of energy dense glucose. In the new study, researchers developed an artificial process that uses the same green light portion of the visible light spectrum used by plants during natural photosynthesis to convert CO_2 and water to fuel in conjuction with electron-rich gold nanoparticles that serve as a catalyst.

Best Practices: A wound is a disruption of the normal structure and function of the skin and skin architecture. To ensure proper healing, the wound bed needs to be well vascularized, free of devitalized tissue, clear of infection, and moist. Wound dressings should eliminate dead space, control exudate, prevent bacterial overgrowth, ensure proper fluid balance, be cost-efficient, and be manageable for the patient and/or nursing staff.

"There is nothing in the world so irresistibly contagious as laughter and good humor." so ease your mind with some light humour in our **Relax Mood section**.

Looking forward for your feedback & suggestions.

Methods for *In vitro* Evaluation of Antimicrobial Activity (Issue II)

Two million people in India die each year due to infectious diseases. There is a need to integrate medicine and innovative technology in our public health system to provide rapid, efficient, accurate, and cost-effective results for identification and antimicrobial susceptibility testing (AST) of pathogens. Automated AST systems can aid in rapid diagnosis of bacterial pathogens.

Antimicrobial resistance has emerged as one of the most-significant health care problems of the new millennium, and the clinical microbiology laboratory plays a central role in optimizing the therapeutic management of patients with infection.

Introduction

A limited number of methods for antimicrobial susceptibility testing (AST) of medically important microorganisms have survived the maturation of modern diagnostic clinical microbiology. Surprisingly, one of these is the disk diffusion method first published in 1966 and the various alterations thereof. The most important outcome of any AST is the rapid and reliable prediction of antimicrobial success in the treatment of infection.

A variety of laboratory methods can be used to evaluate or screen the in vitro antimicrobial activity of a compound. The most known and basic methods are the disk-diffusion and broth or agar dilution methods. Other methods are used especially for antifungal testing, such as poisoned food technique. To further study the antimicrobial effect of an agent in depth, time-kill test and flow cytofluorometric methods are recommended, which provide information on the nature of the inhibitory effect (bactericidal or bacteriostatic) (time-dependent or concentration-dependent) and the cell damage inflicted to the test microorganism. Owing to the new attraction to the properties of new antimicrobial products like combating multidrug-resistant bacteria, it is important to develop a better understanding of the current methods available for screening and/or quantifying the antimicrobial effect of a pure compound for its application in human health, agriculture and environment. Therefore, in this review, the techniques for evaluating the in vitro antimicrobial activity were discussed in detail.

Dilution methods

Dilution methods are the most appropriate ones for the determination of MIC values, since they offer the possibility to estimate the concentration of the tested antimicrobial agent in the agar (agar dilution) or broth medium (macrodilution or micro-dilution). Either broth or agar dilution method may be used to quantitatively measure the in vitro antimicrobial activity against bacteria and fungi. MIC value recorded is defined as the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of the microorganism tested, and it is usually expressed in µg/ml or mg/L. There are many approved guidelines for dilution antimicrobial susceptibility testing of fastidious or non-fastidious bacteria, yeast and filamentous fungi. The most recognized standards are provided by the CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). As advised, these guidelines provide a uniform procedure for testing that is practical to perform in most clinical microbiology laboratories. The development of such methodologic standards does not guarantee the clinical relevance of such testing. Nevertheless, it does allow the bioassay to be performed in a standardized approach in order to evaluate the clinical relevance of results.

a. Broth dilution method

Broth micro or macro-dilution is one of the most basic antimicrobial susceptibility testing methods. The procedure involves preparing two fold dilutions of the antimicrobial agent (e.g.1, 2, 4, 8, 16 and 32 mg/mL) in a liquid growth medium dispensed in tubes containing a minimum volume of 2mL (macrodilution) or with smaller volumes using 96-well microtitration plate (microdilution) (Fig. 1). Then, each tube or well is inoculated with a microbial inoculum prepared in the same medium after dilution of standardized microbial suspension adjusted to 0.5 McFarland scale (Fig. 2 and 3). After well-mixing, the inoculated tubes or the 96-well microtitration plate are incubated (mostly without agitation) under suitable conditions depending upon the test microorganism. The experimental methodology to perform accurately the microdilution is schematized in Fig. 4.

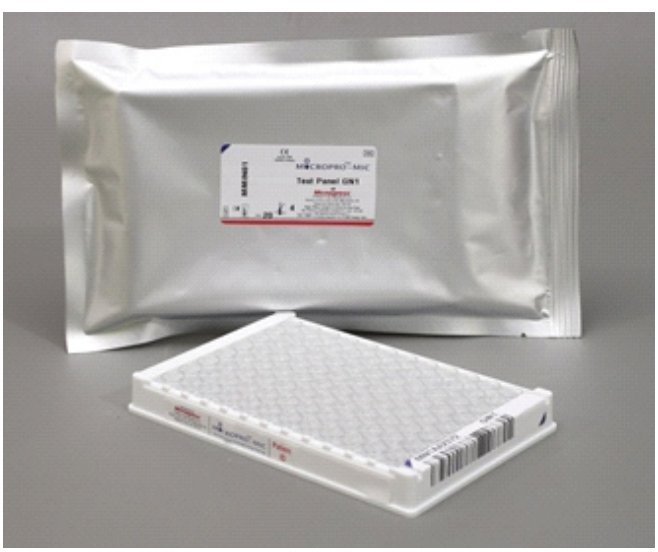


Fig no. 1: 96-well microtitration plate used for Microdilution (Micropro™-MIC)

The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in tubes or micro-dilution wells as detected by the unaided eye. Unlike micro-dilution method, the main disadvantages of the macrodilution method are the tedious, manual undertaking, risk of errors in the preparation of antimicrobial solutions for each test, and the comparatively large amount of reagents and space required. Thus, the reproducibility and the economy of reagents and space that occurs due to the miniaturization of the test are the major advantages of the microdilution method. Nevertheless, the final result is significantly influenced by approach, which must be carefully controlled if reproducible results (intra laboratory and inter laboratory) are to be attained. For the determination of MIC

endpoint, viewing devices can facilitate reading microdilution tests and recording results with high ability to discern growth in the wells. Moreover, several colorimetric methods based on the use of dye reagents have been developed. Tetrazolium salts, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 2,3-bis {2-methoxy-4-nitro-5-[(sulfenylamino) carbonyl]-2H-tetrazolium-hydroxide} (XTT), are often used in the MIC endpoint determination for both antifungal and antibacterial microdilution assays. The Alamar blue dye (resazurin), an effective growth indicator, can also be used for this purpose.

It is well known that the inoculum size, the type of growth medium, the incubation time and the inoculum preparation method can influence MIC values. Therefore, broth dilution has been standardized by CLSI for testing bacteria that grow aerobically, yeast and filamentous fungi. The EUCAST broth dilution method is principally similar to that of CLSI with modifications usually concerning some of the test parameters such as inoculum preparation, inoculum size, and the MIC reading method which is visual in CLSI assay and spectrophotometric in EUCAST guidelines.

As regards to the conidium and spores forming fungi, the microdilution standardized by CLSI involves an inoculum of spores adjusted spectrophotometrically to 0.4×10^{4}–5×10^{4} CFU/mL. However, in the EUCAST assay, the inoculum can be adjusted to (2–5) × 10^{5} CFU/mL by haemocytometer counting. Numerous studies showed the importance of inoculum preparation by haemocytometer counting for reproducible and suitable preparation independent of the color and size of conidia.

The determination of minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC), also known as the minimum lethal concentration (MLC), is the most common estimation of bactericidal or fungicidal activity. The MBC is defined as the lowest concentration of antimicrobial agent needed to kill 99.9% of the final inoculum after incubation for 24 hours under a standardized set of conditions described in document of CLSI, in which the MBC can be determined after broth macrodilution or microdilution by subculturing a sample from wells or tubes, yielding a negative microbial growth after incubation on the surface of non-selective agar plates to determine the number of surviving cells (CFU/mL) after 24 hours of incubation. The bactericidal endpoint (MBC) has been subjectively defined as the lowest concentration, at which 99.9% of the final inoculum is killed. MFC is also defined as the lowest concentration of the drug that yields 98%–99.9% killing effect as compared to the initial inoculum. Several studies have been carried out for evaluation of different test parameters for determination of MFC of various drugs against Candida isolates, Aspergillus and other molds.

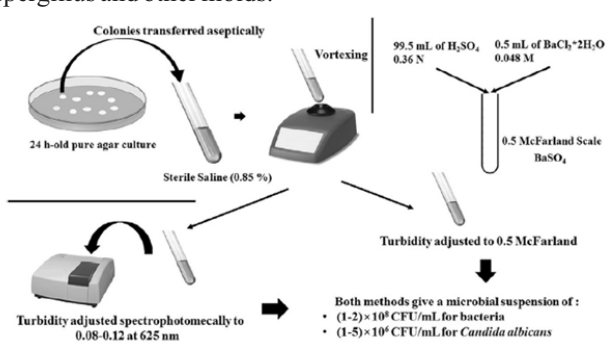
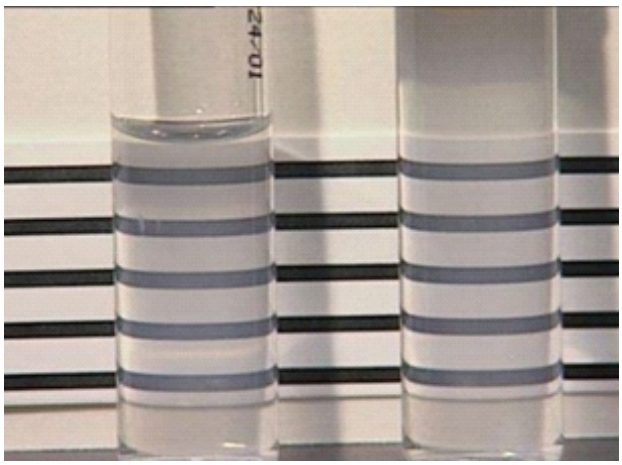


Fig no. 2: 0.5 McFarland microbial inoculum preparation by the direct colony suspension as recommended by CLSI guidelines



(A)

(B)

Fig no. 3: 0.5 McFarland- (A) Barium Sulfate solution that equals the turbidity of 10^{8} Bacteria/mL is used to comparison against a card with a white background and contrasting black lines. (B) a photometric device Called McFarland Reader.

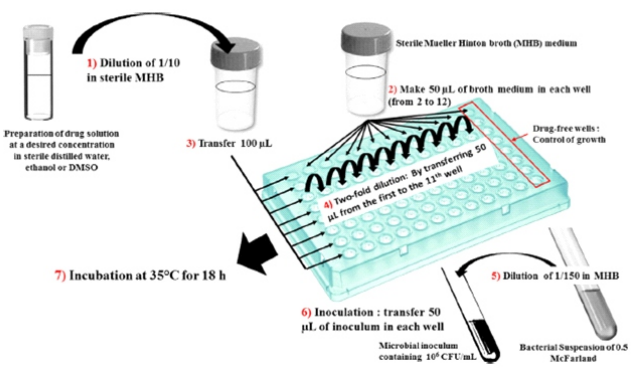


Fig no. 4: Broth microdilution for antibacterial testing as recommended by CLSI protocol.

b. Agar dilution method

The agar dilution method involves the incorporation of varying desired concentrations of the antimicrobial agent into an agar medium (molten agar medium), habitually using serial two-fold dilutions, followed by the inoculation of a defined microbial inoculum on to the agar plate surface. The MIC end point is

recorded as the lowest concentration of antimicrobial agent that completely inhibits growth under suitable incubation conditions (Table1). This technique is suitable for both antibacterial and antifungal susceptibility testing. If multiple isolates are being tested against a single compound, or if the compound tested masks the detection of microbial growth in the liquid medium with its coloring, agar dilution method is often preferred to broth dilution for the MIC determination. Now a days, commercially produced inoculum replicators are available and can transfer between 32 and 60 different bacterial inocula to each agar plate. Agar dilution is often recommended as a standardized method for fastidious organisms such as anaerobes and *Helicobacter* species. It has been also used for antifungal agent drugs combinations against *Candida* sp., *Aspergillus*, *Fusarium* and dermatophytes. This method presents a good correlation with Etest mostly for antibacterial testing against both Gram-positive and Gram-negative bacteria. Moreover, category comparisons of agar dilution, disk-diffusion and broth microdilution methods give excellent results.

Table 1: Culture media, microbial inoculum size and incubation conditions for antimicrobial susceptibility testing methods as recommended by CLSI

Methods	Microorganism	Growth medium	Final inoculum size	Incubation temperature(°C)	Incubation time(h)
Disk-diffusion method	Bacteria	MHA	(0.5 McFarland) $(1–2)\times10^8$ CFU/mL	35±2	16–18
	Yeast	MHA+GMB*	(0.5 McFarland) $(1–5)\times10^6$ CFU/mL	35±2	20–24
	Molds	Non-supplemented MHA	$(0.4–5)\times10^6$ CFU/mL	-	-
Broth microdilution	Bacteria	MHB	5×10^5 CFU/mL	35±2	20
	Yeast	RPMI 1640**	$(0.5–2.5)\times10^3$ CFU/mL	35	24–48
	Molds	RPMI 1640**	$(0.4–5)\times10^4$ CFU/mL	35	48 for most fungi
Broth macrodilution	Bacteria	MHB	5×10^5 CFU/mL	35±2	20
	Yeast	RPMI 1640**	$(0.5–2.5)\times10^3$ CFU/mL	35	46-50
	Molds	RPMI 1640**	$(0.4–5)\times10^4$ CFU/mL	35	48 for most fungi
Agar dilution	Bacteria	MHA	10^4 CFU/spot	35±2	16-20
Time-kill test	Bacteria	MHB	5×10^5 CFU/mL	35±2	0, 4, 18, and 24

MHA: Mueller Hinton Agar.
MHB: Mueller Hinton Broth.
*GMB: the medium was supplemented with 2% glucose and 0.5mg/mL methylene blue.
**RPMI 1640: Roswell Park Memorial Institute medium (with glutamine, without bicarbonate, and with phenol red as a pH indicator) was 1640, buffered to pH 7.0 with MOPS (morpholine propane sulfonic acid) at 0.165M.

Time-kill test (time-kill curve)

Time-kill test is the most appropriate method for determining the bactericidal or fungicidal effect. It is a strong tool for obtaining information about the dynamic interaction between the antimicrobial agent and the microbial strain. The time-kill test reveals a time-dependent or a concentration dependent antimicrobial effect. For bacteria, this test has been well standardized and described in document of CLSI. It is performed in broth culture medium using three tubes containing a bacterial suspension of 5×10^5 CFU/mL. The first and the second tubes contain the molecule or the extract tested usually at final concentrations of 0.25 MIC and 1 MIC, and the third one is considered as the growth control. The incubation is done under suitable conditions for varied time intervals (0, 4, 6, 8, 10, 12 and 24 h). Then, the percentage of dead cells is calculated relatively to the growth control by determining the number of living cells (CFU/mL) of each tube using the agar plate count method. Generally, the bactericidal effect is obtained with a lethality percentage of 90% for 6h, which is equivalent to 99.9% of lethality for 24h. In addition, this method can be used to determine synergism or antagonism between drugs (two or more) in combinations. Similarly, several antifungal substances were studied by this method.

Thin-layer chromatography (TLC) – bioautography

In 1946, Goodall and Levi combined paper chromatography method (PC) with contact bioautography to detect different penicillins for their determination. Thereafter, Fischer and Lautner introduced TLC in the same field. This technique combines TLC with both biological and chemical detection methods. Several works have been done on the screening of organic extracts, mainly plant extracts, for antibacterial and antifungal activity by TLC–bioautography. Three bioautographic techniques, i.e., agar diffusion, direct bioautography and agar overlay assay, have been described for the investigation of antimicrobial compounds by this approach.

a. Agar diffusion

Also known as agar contact method, it is the least-employed one of the techniques. It involves the transfer by diffusion of the

antimicrobial agent from the chromatogram (PC or TLC) to an agar plate previously inoculated with the microorganism tested. After some minutes or hours to allow diffusion, the chromatogram is removed and the agar plate is incubated. The growth inhibition zones appear in the places, where the antimicrobial compounds contact with the agar layer.

b. Direct bioautography

Direct bioautography is the most applied method among these three methods. The developed TLC plate is dipped in to or sprayed with a microbial suspension. Then, bioautogram is incubated at 25°C for 48 h under humid condition. For visualization of the microbial growth, tetrazolium salts are frequently used. These salts undergo a conversion to corresponding intensely colored formazan by the dehydrogenases of living cells. p-Iodonitrotetrazolium violet is the most suitable detect ion reagent. These salts are sprayed onto the bioautogram, which is reincubated at 25°C for 24h or at 37°C for 3–4 h. The Mueller Hinton Broth supplemented with agar has been recommended to give a medium sufficient fluid to allow a best adherence to the TLC plate and maintain appropriate humidity for bacterial growth. Direct bioautography may be utilized with either fungi or bacteria. It is the easiest technique for the detection of antifungal substances, and also gives consistent results for spore-producing fungi such as *Aspergillus*, *Penicillium* and *Cladosporium*. For bacteria, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* strains are frequently used to identify antibacterial compounds.

c. Agar overlay bioassay

Also known as immersion bioautography, it is a hybrid of the both previous methods. TLC plate is covered with a molten seeded agar medium. In order to allow a good diffusion of the tested compounds in to the agar medium, the plates can be placed at low temperature for few hours before incubation. After incubation under suitable conditions depending upon the test microorganism, staining can be made with tetrazolium dye. Like direct bioautography, this method can be applied to all microorganisms such as *Candida albicans* and molds. It provides well-defined growth inhibition zones and is not sensitive to contamination. Overall, TLC–bioautography is a simple, effective and in- expensive technique for the separation of a complex mixture, and at the same time, it localizes the active constituents on the TLC plate. Therefore, it can be performed both in sophisticated laboratories and small laboratories which only have access to a minimum of equipment. Although having sophisticated on- line high performance liquid chromatography coupled bioassay, which is becoming increasingly popular as the method of choice for a final clean-up of extractive fractions to obtain pure compounds, the TLC–bioautography offers a rapid technique for the screening of a large number of samples for bioactivity and in the bioactivity guided fractionation. It can be used for detection of antimicrobials in environmental and food samples as well as for searching for new antimicrobial drugs.

Automated Endoscope Reprocessors

ABSTRACT
European Society of Gastrointestinal Endoscopy (ESGE) and the European Society of Gastroenterology Nurses and Associates (ESGENA) sets standards for the reprocessing of flexible endoscopes and endoscopic devices used in gastroenterology. An expert working group of gastroenterologists, endoscopy nurses, chemists, microbiologists, and industry representatives provides updated recommendations on all aspects of reprocessing in order to maintain hygiene and infection control.

5.5 Principles for the Use of Process Chemicals
RECOMMENDATION
Process chemicals must be compatible with endoscopes and endoscope components, endoscopic accessories, and the reprocessing equipment. (e.g. EWDs).

RECOMMENDATION
Reprocessing should employ single-use chemicals only.

RECOMMENDATION
Detergents should be compatible with the applied disinfectant and any detergent residue carried over into the disinfectant solution should not impair the microbiological efficacy of the disinfectant.

RECOMMENDATION
Deposition of process chemicals should be avoided.
Process chemicals used for endoscope reprocessing are designed, tested, and manufactured according to the European Medical Device Directive and their claimed activity has been demonstrated:

- Detergents are class I medical device products recognized by the CE sign on the label.
- Disinfectants are class IIb medical device products recognized by the CE sign plus a four-digit number on the label.

Material compatibility tests are performed on test pieces or on complete endoscopes using the detergent and the disinfectant alone and in combination. Manufacturers of process chemicals, endoscopes, and EWDs should provide information about material compatibility. Slight cosmetic changes with no negative impact on the functionality of the endoscopes can be accepted. Any kind of deposition can be of concern for microbiological growth.

5.5.1 Detergents
RECOMMENDATION
Detergent solutions applied for manual cleaning should not be reused.

RECOMMENDATION
Detergent solutions with a claim of antimicrobial activity (for staff and environment protection) can be reused, and should be freshly prepared at least on a daily basis. The frequency of changing these detergent solutions depends on the number of reprocessed endoscopes. However, if a solution is visibly dirty, it must be changed immediately.

RECOMMENDATION
Detergents containing aldehydes should not be used for the manual cleaning step, as they denature and coagulate protein (fixation).

Detergents can be divided into two main groups:

- Those with an enzymatic and/or alkaline booster.
- Those containing antimicrobial active substances.

Detergents containing antimicrobial active substances are used only for the bedside and the manual cleaning steps.

5.5.2Disinfectants
RECOMMENDATION
Disinfectants used for reprocessing flexible endoscopes should be tested according to the European Standard EN14885. The required disinfection efficacy must be:

- Bactericidal
- Mycobactericidal
- Fungicidal
- Virucidal against enveloped and non-enveloped viruses.

RECOMMENDATION
Disinfectant activity should be demonstrated under "use" conditions in the presence of interfering substances, according to EN ISO 15883.
The EN 14885 standard specifies the requirements for disinfection efficacy and the test protocols that should be applied to prove the efficacy. The EN ISO 15883 standard requires additional tests under use conditions (e. g. of temperature and time) to demonstrate that there is no negative effect from carryover of residues from previous cycles (residues from the load or from the detergent).
Disinfectants containing oxidizing substances or aldehydes act by chemical reactions with microorganisms and they are broadly efficacious against them.
Alcohols, phenols, and quaternary ammonium compounds are not recommended for endoscope disinfection as they do not show the required efficacy against all relevant microorganisms.
In the United Kingdom and France, national guidelines recommend against using aldehyde- and alcohol-based disinfectants in endoscope reprocessing because of their protein fixative properties.

5.5.3Rinse Aid
RECOMMENDATION
If a rinsing aid is used to improve drying of endoscopes, its toxicological characteristics should be assessed according to ISO 10993 – 1 (Biological Assessment of Medical Devices) as this substance remains on endoscope surfaces.

5.5.4Combination of products from different manufacturers
RECOMMENDATION
Detergents and disinfectants as well as rinsing aids should only be used and combined in compliance with the recommendations of the manufacturers of endoscopes, EWDs, and process chemicals.
The combination of different product groups for cleaning and disinfection could cause compatibility problems. Therefore, the manufacturers' recommendations must be followed at all times. Interactions can cause a change of color of endoscope surfaces and depositions or sedimentation on surfaces of endoscopes and inside EWDs. For example, the combination of glutaraldehyde with detergents containing antimicrobial substances based on

amine compounds may cause colored residues as a result of chemical interaction. Any kind of deposition can be of concern regarding microbiological growth.

5.5.5 Change of process chemicals

If an endoscopy department plans to change detergents and/or disinfectants:

- The user should consult the persons/department responsible for infection control and occupational health, as well as the relevant personnel of the clinical.
- service provider.
- Manufacturers of endoscopes, EWDs, and process chemicals must provide compatibility evidence.
- Any necessity for requalification of the reprocessing procedure/EWD must be clarified.
- Staff must be trained in the changed reprocessing procedure taking into account the new products.

Prior to the use of different process chemistry, it is strongly recommended that a requalification of the process should be performed in order to demonstrate efficacy. The qualification of EWD processes should be performed according to the requirements of EN ISO 15883-4. Unauthorized use of chemical products may invalidate guarantees and/or service contracts.
Staff training must include information about contact time, concentration of products, and personal protection measures.

6. Reprocessing of endoscopes

6.1 General Considerations

RECOMMENDATION

Each endoscopy unit should have department-specific standard operating procedures based on manufacturers' IFUs.

RECOMMENDATION

Detailed instructions should be given for the treatment of each of the different types of equipment (including endoscopes) used in the department.

RECOMMENDATION

The reprocessing staff should be aware of the risks and of the importance of each reprocessing process step.

RECOMMENDATION

Department-specific protocols should periodically be updated and archived.

GI endoscopes can have a normal bacterial load of 10^{8-10} (8 – 10 $\log_{10}$). Standardized automated reprocessing cycles lead to an 8 – 12 $\log_{10}$ reduction in microorganisms. Consequently, the safety margin is very low, at 0 –2 $\log_{10}$. Therefore, it is essential to adhere to the standardized protocols.

The efficacy of endoscope reprocessing depends on the reprocessing staff´s comprehensive knowledge of the construction and function of the equipment. Hence, it is essential to have detailed protocols describing the different steps of reprocessing necessary for each type of endoscope. Reprocessing protocols need to be updated on a regular basis, taking into account, for example, new equipment, technical modifications, and updated guidelines and laws/regulations. Reprocessing staff must be informed accordingly about such changes.

The reprocessing workflow consists of four different phases (Fig. 1):

- Bedside cleaning.
- Manual cleaning at the reprocessing area (including leak testing and brushing of endoscope channels).
- Cleaning and disinfection.
- Drying and storage (if required).

<table>
<tr><td>In the endoscopy procedure room</td><td colspan="4">Bedside Cleaning
rinsing and flushing of all channels
function control</td></tr>
<tr><td></td><td colspan="4">Transport from endoscopy room to reprocessing area
and start of manual cleaning steps within approximately 30 minutes</td></tr>
<tr><td rowspan="9">In the separated reprocessing room

Dirty Side</td><td colspan="4">Manual Cleaning
including manual leakage test,
external and internal cleaning, including brushing</td></tr>
<tr><td colspan="4">Waiting time between manual cleaning and reprocessing in
the EWD should not exceed the duration of one EWD cycle</td></tr>
<tr><td colspan="4">Cleaning and Disinfection</td></tr>
<tr><td>Manual reprocessing</td><td colspan="2">EWD</td><td>ADD</td></tr>
<tr><td>Rinsing ⇩</td><td colspan="2" rowspan="4">All reprocessing steps are performed in the EWD:
▪ integrated leakage test
▪ cleaning
▪ rinsing
▪ disinfection
▪ final rinsing
▪ drying</td><td>Rinsing may be included in some ADDs</td></tr>
<tr><td>Disinfecting ⇩</td><td>Disinfection and final Rinsing are included in all ADDs</td></tr>
<tr><td>Final rinsing ⇩</td><td rowspan="2">Drying is an additional option in some ADDs</td></tr>
<tr><td>Drying or direct use</td></tr>
<tr><td rowspan="5">In the separated reprocessing room

Clean Side</td><td rowspan="5">Sterilization
In the case of medical Indication only</td><td colspan="2">Drying or direct use?</td><td rowspan="5">Direct use</td></tr>
<tr><td colspan="2">Drying</td></tr>
<tr><td>Automated Drying</td><td>Manual drying with compressed air</td></tr>
<tr><td colspan="2">Storage</td></tr>
<tr><td>Storage cabinet with drying function</td><td>Endoscope cabinet without drying function</td></tr>
<tr><td></td><td colspan="4">Transport to next patient</td></tr>
</table>

Fig. 1 – Different methods of endoscope reprocessing. EWD, endoscope washer-disinfector; ADD, automated disinfection device

For safe and effective reprocessing, it is essential to follow all the steps of the reprocessing workflow in a thorough and timely manner. The clinical service provider must document and explain any deviation from their specific reprocessing workflow.

RECOMMENDATION
Endoscope reprocessing should always be performed immediately after finishing the procedure, regardless of where the endoscopic procedure is performed.

RECOMMENDATION
The time that elapses between manual cleaning and reprocessing in the EWD should not exceed the time of one EWD cycle.

Cleaning is the most important step in reprocessing. It is impossible to effectively disinfect or even sterilize an inadequately cleaned instrument.

Bedside cleaning and the manual cleaning steps with flushing and brushing of the entire channel systems are the most important steps for the removal of debris, blood, and body fluids. Remaining protein debris can become fixed by drying or by the use of inappropriate chemicals. Biofilm formation is possible if the cleaning and rinsing steps have not been carried out correctly. As some Gram-negative bacteria can undergo cell division every 20 to 30 minutes, it is essential to complete all reprocessing steps quickly, before bacterial growth and debris begin to dry on surfaces. Microorganisms embedded in biofilms are 10 to 100 times more resistant to process chemicals than planktonic (free-floating) microorganisms and are frequently released from biofilms. Therefore, it is important to follow the IFU of the endoscope manufacturer and the national guidelines. Some national guidelines recommend performance of all manual reprocessing steps within 30 minutes after completion of the patient examination. If endoscope reprocessing is delayed, augmented cleaning steps may be considered.
Endoscopes that are immersed into detergent or disinfecting solutions for several hours may be damaged.

6.2 Bedside cleaning
RECOMMENDATION
Bedside cleaning of the endoscope should start immediately after the endoscope has been withdrawn from the patient, in order to:
- Remove debris from external and internal surfaces.
- Prevent any drying of body fluids, blood, or debris.
- Reduce any build-up of bio burden or growth of biofilms.
- Carry out a first check for correct functioning of the endoscope channels.

The insertion tube and critical components (e. g. the distal end of duodenoscopes and echoendoscopes) should be wiped externally with cleaning solution, using a soft, disposable cloth/sponge, and checked for any macroscopic damage.
Typically, air/water channels should be flushed with water from the water bottle. It is important to consider the use of cleaning valves for the air/water channel, according to the manufacturer's IFU.

Before the endoscope is detached from the light source and video processor, detergent solution should be sucked through the instrument/suction channel. European and national guidelines recommend flushing with a volume of 200 – 250mL or for a duration of 10– 20 seconds as a benchmark. Flushing must be continued until clear suction liquid demonstrates the cleanliness of the channel system.

Additional channels should be rinsed/flushed according to the manufacturer's IFU.

The presence of any faults, such as blockages or defects, must be communicated to the reprocessing staff so that they can be addressed appropriately.

6.3 Transport of contaminated equipment
RECOMMENDATION
After completion of bedside cleaning, each precleaned endoscope and its components and accessories should be transported in a closed container, clearly marked as contaminated equipment, to the reprocessing room.

RECOMMENDATION
Such containers should be cleaned and disinfected manually using surface disinfectants or automatically in CSSDs.

Transport in closed containers avoids contamination of the environment and third parties.

Even if several endoscopes are used during one procedure, each endoscope should be transported in a separate container, in order to avoid any damage and to enable separation from other equipment. In the United Kingdom, the endoscope and its valves stay together as a traceable unique set and the valves should not be used with any other endoscope.

6.4 Manual cleaning in the reprocessing area
6.4.1Leak Test
RECOMMENDATION
The manual leak test should be performed according to the manufacturer's IFU, after bedside cleaning but before starting any further cleaning steps.

RECOMMENDATION
The manual leak test should be performed in addition to automated leak tests in the EWD in order to identify any damage at an early stage.

RECOMMENDATION
In the case of any detected leakage, the reprocessing procedure must be interrupted immediately, and repair of the endoscope should be initiated. In such cases, the user should clearly mark the endoscope as “Not disinfected” prior to shipment to the nearest repair centre.

Outbreaks in gastroenterological, bronchoscopic, and cardiological settings showed that damaged parts of endoscopes may become reservoirs for microorganisms that cause cross-contamination and severe infections. Therefore, it is essential that the manual leak test is performed at the start of each reprocessing cycle.

6.4.2Equipment for Manual Cleaning
RECOMMENDATION
During manual cleaning stages, only single-use cleaning solutions, brushes and other cleaning devices (such as sponges and cloths) should be used. This is in order to:
- Ensure maximum and standardized effectiveness ofcleaning.

- Avoid any damage to endoscope components.
- Reduce any tissue carry-over and cross-contamination.

RECOMMENDATION
The endoscopes should be placed into sinks of appropriate size and fully immersed in detergent solution before brushing activities are started.

RECOMMENDATION
The size (length and diameter) and type of cleaning brush should appropriately match the size and type of the endoscope channel to ensure contact with channel walls, and access to all small/narrow lumens.

RECOMMENDATION
Purpose-designed brushes should be used for cleaning of critical endoscope components (such as the elevator mechanism of duodenoscopes and echoendoscopes), according to the manufacturer's IFU.

RECOMMENDATION
Special connectors and cleaning devices should be available for each type of endoscope used in a department. Reusable connectors should be cleaned and maintained according to standardized reprocessing protocols and according to the manufacturer's IFU.

Single-use brushes ensure a standardized cleaning quality as these have undamaged bristles without any tissue remaining from previous examinations. Consequently, European and national guidelines recommend use of single-use brushes only.

Damage to fragile endoscope components may be caused by damaged cleaning brushes. Following outbreaks of carbapenem-resistant Enterobacteriaceae (CRE) infections in the United States, reviews and surveys considered the off-label use of cleaning brushes that may have promoted the outbreaks. The outbreaks stopped when the departments changed to single-use brushes. Reusable brushes may carry risks from insufficiently cleaned bristles and from kinks that may damage internal surfaces of endoscopes. In order to avoid any cross-contamination, reusable brushes must be reprocessed between each endoscope reprocessing.

Various types of brushes are available for different channel diameters and for special endoscope components such as valves, ports, or distal tips. The different endoscope channels and components should be reprocessed according to the manufacturer's IFU.

All types of duodenoscopes require meticulous manual cleaning, since crevices behind the elevator cannot easily be reached with conventional brushes. Manufacturers provide purpose-designed small brushes and reprocessing recommendations, which should be incorporated into existing department specific reprocessing protocols. In addition, various design improvements for endoscopes have been developed in recent years, including single-use components for distal tips and removable elevator mechanisms that can be autoclaved. ESGE and ESGENA, as well as national bodies and professional societies, have also published statements focusing on CRE infections and duodenoscope reprocessing.

All endoscopes are supplied with the appropriate cleaning adapters that ensure appropriate access to and rinsing of all accessible endoscope channels. These cleaning adapters should be used in manual cleaning steps according to the manufacturer's IFU.

6.4.3Manual Cleaning Steps
RECOMMENDATION
Thorough cleaning should cover all external surfaces, critical components (e. g. elevator mechanism, valves)and all accessible endoscope channels, in line with the manufacturer's IFU.

RECOMMENDATION
Special attention should be given to complex endoscopes such as duodenoscopes and echoendoscopes.

RECOMMENDATION
Detergent concentrations and contact times of the detergent should follow its manufacturer's recommendations.

Thorough manual cleaning with detergent is the most important step of the endoscope reprocessing procedure as any debris that remains may impair the efficacy of subsequent reprocessing steps and may support the formation of biofilms.

Cleaning steps for the endoscope include:
- Full immersion of the endoscope in detergent solution.
- Cleaning of all external surfaces, valve ports, channel openings, and distal tips (including the elevator mechanism of duodenoscopes or the balloon of echoendoscopes), using a soft disposable cloth, sponges, and/or purpose-designedbrushes.
- Brushing of all accessible channels using flexible, purpose-designedsingle-use brushes, until there is no visible debris. The direction and order of brushing should be considered, according to the manufacturer's IFU.
- Flushing of all lumens in order to remove organic material(blood, tissue, stool, etc.) after brushing. Endoscope type-specificcleaning adapters must be used in order to access all channels.
- Even if they have not been used during the endoscopic procedure, all the auxiliary water channels, wire channels, and balloon channels (in echoendoscopes and probes) must be flushed with detergents. Because of the capillary effect, all the endoscope channels become contaminated and partly filled with fluids/debris even when they have not been directly used in the endoscopic procedure.
- Flushing of the endoscope channels also confirms the correct functioning and patency of the endoscope channels.

There is a clear trend toward single-use endoscope components(e.g. biopsy ports, valves, distal caps). If these detachable endoscope components are reusable, they must be cleaned using dedicated brushes, according to the manufacturer's IFU.

During manual cleaning it is important to follow the detergent contact time, temperature, and concentration as recommended by its manufacturer in order to ensure the detergent's efficacy. Flushing of endoscope channels can be done manually or can be supported by automated flushing/rinsing devices.

All guidelines emphasize the thorough cleaning of endoscope channels. French guidelines recommend double cleaning. Multiple cleaning procedures may show positive reprocessing results. However, it is difficult to exactly calculate the optimal number of brushing cycles, as contamination varies greatly from patient to patient.

RECOMMENDATION

Fresh water (drinking water of defined quality, without any pathogens) should be used as the rinsing solution for each endoscope.

RECOMMENDATION

It is recommended to use a separate rinsing sink of appropriate size in addition to the cleaning sink.

Rinsing of external surfaces and all channels removes residual debris and detergent to a level that avoids any critical interactions in the subsequent reprocessing phases.

Depending on the detergent used, this rinsing step may also be performed in the EWD as a first rinse before starting the automated cleaning and disinfection cycles.

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To be continued……..

Lynn Margulis

Lynn Margulis (March 15, 1938 – November 22, 2011) was a biologist and university professor who pioneered important concepts in the fields of cell biology and microbial evolution. She perhaps is best known for her contributions to the endosymbiotic theory, which is now generally accepted for how certain eukaryotic organelles were formed.

The endosymbiotic theory concerns the origins of mitochondria and plastids (e.g. chloroplasts). According to this theory, these organelles originated as separate prokaryotic organisms that were taken inside another cell as endosymbionts. Both the host cells and the symbionts would have received advantages from the symbiotic relationship, and this would have eventually led to their integration. The fact that mitochondria and plastids have their own DNA and ribosomes is one of many supports for this theory. Mitochondria are considered to have developed from proteobacteria and chloroplasts from cyanobacteria.

Margulis saw symbiogenesis—the development of new organisms, organelles, and so forth from the merging of two separate organisms—as a fundamental factor in creating genetic variation (more so than mutation) and as a primary force in evolution. In general, and in contrast to neo-Darwinism, Margulis holds that "Life did not take over the globe by combat, but by networking" (Margulis and Sagan 1986)—in other words, more by cooperation than via Darwinian competition. Margulis was also an important collaborator with James Lovelock in developing the concepts related to the Gaia hypothesis. The Gaia hypothesis is a class of scientific models of the geo-biosphere in which life as a whole fosters and maintains suitable conditions for itself by helping to create a favorable environment on Earth for its continuity. The Gaia hypothesis addresses the remarkable harmony seen between biotic and abiotic elements on Earth; similarly, the endosymbiotic theory touches on the harmony among biotic elements.

Theoretical contributions

In 1966, as a young faculty member at Boston University, Margulis wrote a theoretical paper titled The Origin of Mitosing Eukaryotic Cells (Sagan 1967). The paper, however, was "rejected by about fifteen scientific journals," Margulis recalled (Brockman 1995). It was finally accepted by The Journal of Theoretical Biology and is considered today a landmark in modern endosymbiotic theory.

Although this article draws heavily on symbiosis ideas first put forward by scientists in the mid-nineteenth century, as well as the early twentieth century work of Merezhkovsky (1905) and Wallin (1920), Margulis's endosymbiotic theory formulation is the first to rely on direct microbiological observations (as opposed to paleontological or zoological observations, which were previously the norm for new works in evolutionary biology). Weathering constant criticism of her ideas for decades, Margulis is known for her tenacity in pushing her theory forward, despite the opposition she faced at the time.

The underlying theme of endosymbiotic theory, as formulated in 1966, was interdependence and cooperative existence of multiple prokaryotic organisms; one organism engulfed another, yet both survived and eventually evolved over millions of years into eukaryotic cells. Her 1970 book, Origin of Eukaryotic Cells, discusses her early work pertaining to this organelle genesis theory in detail. Currently, her endosymbiotic theory is recognized as the key method by which some organelles have arisen and is widely accepted by mainstream scientists. The endosymbiotic theory of organogenesis gained strong support in the 1980s, when the genetic material of mitochondria and chloroplasts was found to be different from that of the cell's nuclear DNA (Sehi 2001).

Symbiogenesis is the general term used for the merging of two separate organisms to form a single new organism. In Acquiring Genomes: A Theory of the Origins of Species, published in 2002, Margulis argues that symbiogenesis is a primary force in evolution; that is, symbiotic relationships between organisms of often different phyla or kingdoms are the driving force of evolution.

This concept challenges a central tenet of neodarwinism that inherited variation mainly comes from random mutations. According to Margulis' theory, acquisition and accumulation of random mutations are not sufficient to explain how inherited variations occur. Rather, Margulis argues that genetic variation occurs mainly as the result of the transfer of nuclear information between organisms. New organelles, bodies, organs, and species arise from symbiogenesis, evolving primarily through relationships between organisms, involving the fusion of genomes.

Whereas the classical interpretation of evolution, (neo-Darwinism), emphasizes competition as the main force behind evolution, Margulis emphasizes cooperation as the most important factor in the development of life.

While Margulis' organelle genesis ideas are widely accepted, her

further hypothesis that symbiotic relationships are a current method of introducing genetic variation is not considered to be mainstream in evolutionary theory. Nonetheless, examination of the results from the Human Genome Project lends credence to an endosymbiotic theory of evolution—or at the very least it positions Margulis's endosymbiotic theory to serve as catalyst for generating ideas about the origins of the current composition of the human genome. From the perspective of the endosymbiotic theory, significant portions of the human genome are apparently either bacterial or viral in origin—with some clearly being ancient insertions, while others are more recent in origin. This strongly supports the idea of the close association of organisms, with symbiotic, or more likely parasitic relationships, being a driving force for genetic change in humans, and likely all organisms.

Overall, while many ecologists agree with Margulis's emphasis on symbiosis as a driving force of evolution, this idea has little support from other evolutionary biologists. They see little evidence that symbiogenesis has had a major impact on eukaryotic life, or that much of its diversification can be attributed to it, other than the two examples of mitochondria and chloroplasts. It is a fundamental principle of classical neo-Darwinism, or population genetics theory, that mutations arise one at a time and either spread through the population or not, depending on whether they offer an individual fitness advantage. Nevertheless, the neo-Darwinist perspective remains vulnerable to challenges like that of Margulis because its experimental support comes overwhelmingly from the laboratory, not from the wild. It is understood clearly how artificial selection works in the laboratory, but there is legitimate controversy over whether nature's laboratory works in just this way. Indeed, genome mapping techniques have revealed that family trees of the major taxa appear to be extensively cross-linked—possibly due to lateral transfer of genes carried by bacteria, as Margulis predicted.

It should be noted that while the endosymbiotic theory has often been presented as being fundamentally opposed to the neo-Darwinian model, the two theories are not incompatible. Nonetheless, Margulis holds a generally negative view of neo-Darwinism, as she believes that history will ultimately judge the theory as "a minor twentieth century religious sect within the sprawling religious persuasion of Anglo-Saxon Biology" (Mann 1991). She also believes that proponents of the standard theory, "wallow in their zoological, capitalistic, competitive, cost-benefit interpretation of Darwin—having mistaken him… Neo-Darwinism, which insists on (the slow accrual of mutations), is a complete funk" (Mann 1991).

Margulis' present day efforts, in the form of books and lectures, strongly stress a symbiotic—and cooperative—relationship between all organisms and a strong leaning toward Gaia theory. Her advocacy outside the realm of biology and toward more sociopolitical ends has been criticized by more mainstream scientists—somewhat similar to criticisms aimed toward Carl Sagan's latter day ideas.

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Santa - My wife died yesterday...
I'm trying to cry but tears are not come out, what to do?

Banta - No Problem.
Just Imagine she Came Back.

www.olaalaa.com

Teacher : Who is the President of Iraq ?

Johnny : I don't know Miss

Teacher : You need to focus more on your studies.

Johnny : Please Miss, can I ask a question ?

Teacher : Yes.

Johnny : Do U know Angela ?

Teacher : No, why ?

Johnny : You need to focus more on your husband!

- 😂😂😂 -

Teacher : Why your paper is blank?

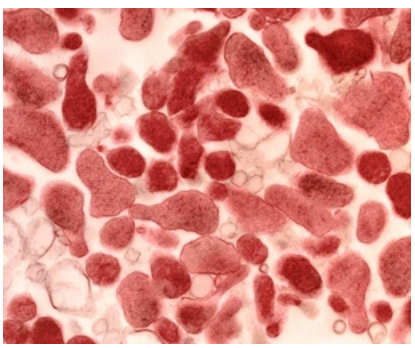
Student : Sometimes, silence is the best answer.

Boy:
"What's your age...?"
Girl:
"We don't reveal our age to boys...!"
Boy:
"What's your email address...?"
Girl:
"pooja_1988@gmail.com"

LOLS ALOT!

Wonderful coffee Customer to waiter: Everyday you charge me money for a cup of coffee. It will be wonderful if you serve me coffee free of cost today. Waiter: Sir, everyday you drink coffee from a filled cup. It will be wonderful if you drink it from an empty cup today. ...

Mycoplasma genitalium: the next sexually transmitted superbug?



Mycoplasma genitalium (*MG*, commonly known as Mgen), is a sexually transmitted small and pathogenic bacterium that lives on the skin cells of the urinary and genital tracts in humans. Mgen is becoming increasingly common. Resistance to multiple antibiotics is occurring, including azithromycin which until recently was the most reliable line treatment. The bacteria was first isolated from urogenital tract of humans in 1981, and was eventually identified as a new species of *Mycoplasma* in 1983. It can cause negative health effects in men and women. It also increases the risk factor for HIV spread with higher occurrences in homosexual men and those previously treated with the azithromycin antibiotics. Specifically, it causes urethritis in both men and women, and also cervicitis and pelvic inflammation in women. It presents clinically similar symptoms to that of *Chlamydia trachomatis* infection and has shown higher incidence rates, compared to both *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in some populations.

Antimicrobial resistance and treatment failures are the biggest challenges

The publication of national treatment guidelines does not usually generate headlines in national newspapers. However, the recent release of draft management guidelines for *Mycoplasma genitalium* infection was accompanied by high profile media coverage suggesting that it is the next sexually transmitted "superbug." So what are the facts behind these headlines, and how concerned should we be?

First isolated in 1981, *M genitalium* is the smallest known self replicating bacterium, but its natural course of infection and importance for public health remain poorly understood. Most infections are probably asymptomatic and have no adverse health outcomes. Nonetheless, evidence that *M genitalium* is associated with serious genitourinary and reproductive health morbidity is accumulating.

In men, an unequivocal association exists with non-gonococcal urethritis, and it is detected in up to 40% of men with persistent and recurrent urethritis. There is some evidence of associations with balanoposthitis but no clear association with prostatitis or epididymitis. A study among men who have sex with men found no association with symptoms of proctitis and rectal infection. In women, a recent meta-analysis found significant associations with a range of clinical syndromes and adverse reproductive health outcomes, including cervicitis, postcoital bleeding, pelvic inflammatory disease, preterm birth, and spontaneous abortion, and a weak association with infertility.

Data on population prevalence are sparse, but a meta-analysis of six studies suggested that the prevalence of *M genitalium* infection ranged from 1.3% to 3.9% and was higher in countries with a low development index. In Britain, a probability sample survey estimated a prevalence of around 1.3% in the sexually active British population aged 16-44 years. In common with many other sexually transmitted infections (STIs), *M genitalium* infection rates can be considerably higher in men who have sex with men, sex workers, and people attending STI clinics, but those infected tend to be older than people with other STIs such as chlamydia; 91% of infected men and 67% of infected women are aged 25 to 44.

Antimicrobial resistance

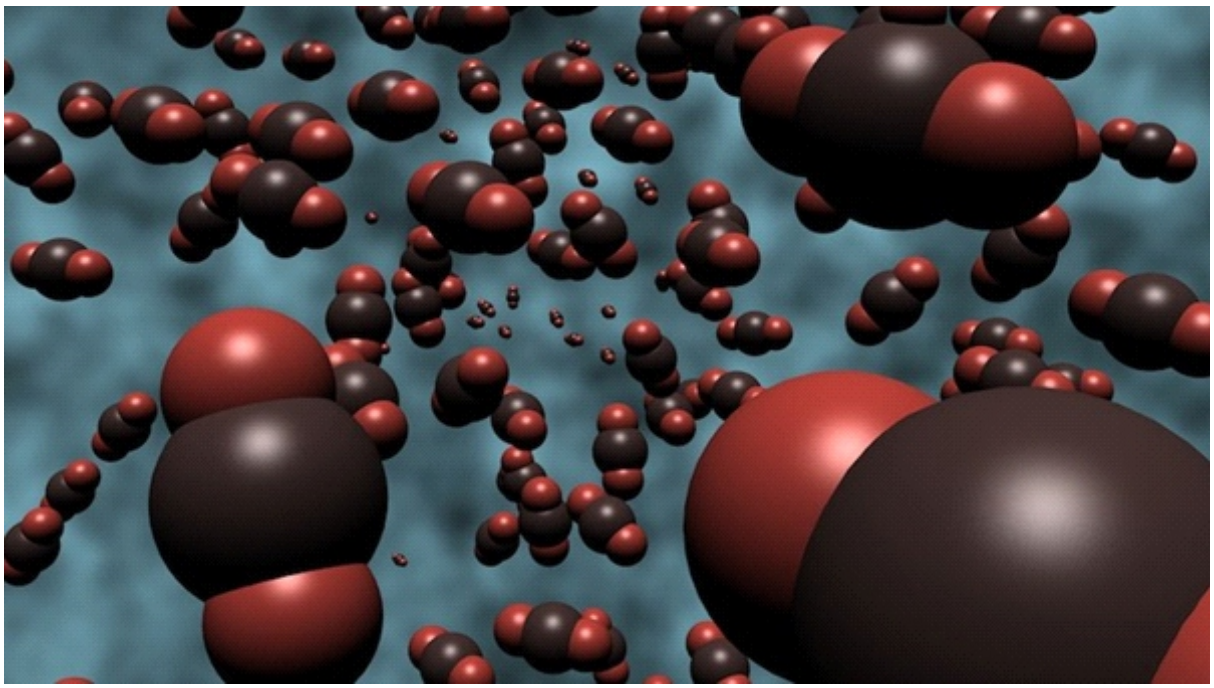
The main concern is *M genitalium*'s increasing resistance to azithromycin and moxifloxacin, the recommended first and second line treatments in Europe, North America, and Australia, especially in the Asia-Pacific region. For example, single nucleotide polymorphisms in region V of the 23S rRNA gene, which confer macrolide resistance, were found in over 60% of *M genitalium* specimens from people attending STI clinics in Australia in 2015. Furthermore, selective pressure can lead to the emergence of macrolide resistance after exposure to suboptimal levels of drug. Mutations in the ParC gene (possibly modified by mutations in GyrA), which confer fluoroquinolone resistance, are also becoming increasingly common. Importantly, resistance markers are highly correlated with treatment failure, especially when the organism load is high. A recent meta-analysis showed the pooled efficacy of a 1 g single dose of azithromycin has declined from 85% in studies conducted before 2009 to 67% in later studies. About 9-12% of *M genitalium* infections may have dual resistance mutations and are therefore unlikely to be effectively treated with azithromycin or moxifloxacin. Treatment options in those who do not respond to first and second line therapy are limited and include extended courses of doxycycline (effective in up to 30% of cases) and pristinamycin, which is not easily available in the UK. Management of such cases will usually require specialist advice from sexual health clinicians and microbiologists.

Such high levels of antimicrobial resistance and treatment failure present challenges not only for managing individual patients but for developing an appropriate public health response. Although nucleic acid amplification tests for *M genitalium* are available, including in multiplex kits testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, routine screening for asymptomatic *M genitalium* infection is not recommended, even in higher risk populations attending STI clinics. Detection of an infection that may not cause harm and may be difficult to cure in people without symptoms could lead to distress, unnecessary treatment, and the selection and spread of resistance.

However, diagnosis and treatment of *C trachomatis* and *N gonorrhoeae* in asymptomatic people attending STI clinics is routine. If these people have undiagnosed coinfections with *M genitalium,* the organism may be exposed to suboptimal macrolide concentrations, potentially selecting macrolide resistance.

The draft UK guideline for *M genitalium* takes a pragmatic view, recommending testing in all men with symptomatic urethritis, women with pelvic inflammatory disease, and their current sexual partners (regardless of symptoms) to reduce the risk of reinfection. Even such limited testing may present practical and financial challenges to service providers and commissioners. But we need to improve the evidence base on the natural course of *M genitalium* infection and develop treatment regimens that support a cost effective public health response to minimise associated harms. Diagnosis of antimicrobial resistance mutations at point of care could also better guide treatment decisions for *M genitalium* and other sexually transmitted organisms.

Artificial photosynthesis transforms carbon dioxide into liquefiable fuels



Chemists at the University of Illinois have successfully produced fuels using water, carbon dioxide and visible light through artificial photosynthesis. By converting carbon dioxide into more complex molecules like propane, green energy technology is now one step closer to using excess CO_2 to store solar energy -- in the form of chemical bonds -- for use when the sun is not shining and in times of peak demand.

Plants use sunlight to drive chemical reactions between water and CO_2 to create and store solar energy in the form of energy-dense glucose. In the new study, the researchers developed an artificial process that uses the same green light portion of the visible light spectrum used by plants during natural photosynthesis to convert CO_2 and water into fuel, in conjunction with electron-rich gold nanoparticles that serve as a catalyst. The new findings are published in the journal *Nature Communications*.

"The goal here is to produce complex, liquefiable hydrocarbons from excess CO_2 and other sustainable resources such as sunlight," said Prashant Jain, a chemistry professor and co-author of the study. "Liquid fuels are ideal because they are easier, safer and more economical to transport than gas and, because they are made from long-chain molecules, contain more bonds -- meaning they pack energy more densely."

In Jain's lab, Sungju Yu, a postdoctoral researcher and first author of the study, uses metal catalysts to absorb green light and transfer electrons and protons needed for chemical reactions between CO_2 and water -- filling the role of the pigment chlorophyll in natural photosynthesis.

Gold nanoparticles work particularly well as a catalyst, Jain said, because their surfaces interact favorably with the CO_2 molecules, are efficient at absorbing light and do not break down or degrade like other metals that can tarnish easily.

There are several ways in which the energy stored in bonds of the hydrocarbon fuel is freed. However, the easy conventional method of combustion ends up producing more CO_2 -- which is counterproductive to the notion of harvesting and storing solar energy in the first place, Jain said.

"There are other, more unconventional potential uses from the hydrocarbons created from this process," he said. "They could be used to power fuel cells for producing electrical current and voltage. There are labs across the world trying to figure out how the hydrocarbon-to-electricity conversion can be conducted efficiently," Jain said.

As exciting as the development of this CO_2-to-liquid fuel may be for green energy technology, the researchers acknowledge that Jain's artificial photosynthesis process is nowhere near as efficient as it is in plants.

"We need to learn how to tune the catalyst to increase the efficiency of the chemical reactions," he said. "Then we can start the hard work of determining how to go about scaling up the process. And, like any unconventional energy technology, there will be many economic feasibility questions to be answered, as well."

The Energy and Biosciences Institute, through the EBI-Shell program, supported this research.

Best Practices in Wound Care Management

INTRODUCTION:

The use of topical antimicrobial agents in wound management

Wound infection can be financially costly to healthcare organisations and can negatively affect quality of life for patients, families and carers, due to pain, malodour, frequent dressing changes, loss of appetite, malaise, or deterioration of glycaemic control in people with diabetes (WUWHS, 2008).

Cases of surgical site infection (SSI)can double length of hospital stay, and healthcare interventions for a patient with an SSI can cost £814 to £6,626, depending on the surgery type and severity of the infection (NICE, 2008). Pressure ulcers can cost an average of £1,214 (category 1)to £14,108 (category IV) each (Dealey etal, 2012). Venous leg ulcers cost the NHS nearly £200 million annually, and diabetic foot ulcers £300 million a year (Posnett and Franks, 2008). Furthermore, it's estimated up to half these wounds will become infected (Posnett and Franks, 2008), which can, in lower limbs, result in amputation —a life-changing outcome desired by neither clinicians nor patients.

Effectively managing and treating wound infection can challenge clinicians, with myriad products and pharmaceutical interventions available. The results of the Health Protection Agency's Point Prevalence Survey on healthcare-associated infections and antimicrobial use estimated the total number of antimicrobials prescribed as 25,942 for 18,219 patients, with the prevalence of antimicrobial drug and device use being 34.7% (HPA, 2011).

However, indiscriminate use of antimicrobials — in particular, antibiotics — has led to the rising prevalence of resistant organisms, with the potential to jeopardise patient outcomes (EWMA, 2013a).

Professor Dame Sallie Davies, Chief Medical Officer for England, recently highlighted the urgency of reviewing the use of antibiotics and antimicrobials. In her annual report, she stated: 'There is a need for politicians in the UK to prioritise antimicrobial resistance as a major area of concern, including it on the national risk register (specifically, the National Security Risk Assessment)' (Davies, 2013). Prof Davies warned that, during the next 50 years, microorganisms' drug resistance will increase, and new strains with resistance to a wide variety of agents will emerge, rendering antimicrobial drugs ineffective. She further suggested development of new antimicrobial agents has declined, leaving fewer options for treating infections (Davies, 2013).

It is therefore essential that clinicians be able to identify wound infections correctly and, when appropriate, choose the right topical antimicrobial and/or systemic antibiotics for treatment, with the goals of preventing/eradicating infection and promoting wound healing.

Effective management and treatment of wound infections is challenging. This document seeks to provide clinicians with a best practice guide on when — and when not — to use topical antimicrobial agents, comprising the following:

- Assessing the patient and wound.
- Biofilms and wound infection.
- Selecting and using topical antimicrobials.
- Considerations in different wound aetiologies.
- Decision-making algorithm for best practice.

SECTION 1: ASSESSING THE PATIENT AND WOUND

Infection-relatedterminology

The WUWHS (2008) identified the presence of microbes in a wound can result in:

- **Contamination**, in which the microbial burden does not increase or cause clinical problems.
- **Colonisation**, in which the microbes multiply, but wound tissues are not damaged; i.e., the wound is on a normal healing trajectory with no clinical evidence of infection.
- **Critical colonisation or localised infection**, in which microbes multiply and the wound moves from benign colonisation to an infected state with impaired healing but without tissue invasion or host immunological response (Moore et al, 2007). However, there is currently no consensus on how to define or identify critical colonisation (EWMA,2013a).
- **Infection (spreading or systemic)**, in which the bacteria multiply, healing is disrupted and deep tissues are damaged. Bacteria might produce localised problems or cause systemic illness (sepsis).

Key Points:

1. Before prescribing any wound products or medications, the clinician must undertake and document a holistic assessment of the patient.
2. Wound infection assessment should include examination of the wound bed and peri wound area, documenting any signs of redness, unexplained pain or malodour.
3. Accurately assess the wound bed to help differentiate viable tissue from non-viable tissue.
4. Several classic signs and symptoms are easily identifiable as wound infection, but not all wounds will exhibit all these signs at any one time.
5. The value of a surface swab is debated.
6. If infection or colonisation is clinically diagnosed, use TIME to develop a wound management plan.
7. Wound healing is a complex and multi faceted process influenced by intrinsic and extrinsic factors, some of which can be controlled.

INTRODUCTION TO INFECTION

All wounds are contaminated with a variety of microorganisms (Stotts, 2004;WUWHS, 2008). In general, these microbes are harmless skin flora naturally found on the skin's surface. Intact skin provides a physical barrier against these microbes; however, the creation of a wound, acute or chronic, damages this defence mechanism, letting microbes enter the body.

Infections have been categorised into those that affect superficial tissues (skin and subcutaneous layer) of the incision and those that affect the deeper tissues (deep incisional or organ-space) (CDC, 2000). See above in infection-related terminology for further terms associated with microbes and their effect on the

wound healing process that will be used throughout the document. Clinicians must be aware of the terminology and confident in their abilities to recognise each.

ASSESSING THE WOUND FOR INFECTION

Before prescribing any wound products or medications, the clinician must undertake and document a holistic assessment of the wound, including examination of the wound bed and periwound area, documenting any signs of redness, unexplained pain or malodour (Ousey and Cook, 2012). However, the assessment should not comprise the wound and its characteristics in isolation but, rather account for a number of factors.

ASSESSING THE PATIENT'S INFECTION RISK

Wound healing is a complex and multifaceted process influenced by intrinsic and extrinsic factors, some of which can be controlled. Patient assessment should encompass the general medical condition, as immune compromised, neonatal and elderly patients are at greater risk of wound infections (White, 2009). In addition, certain chronic medical conditions (e.g. diabetes), medications (e.g. oncology drugs) and lifestyle factors (e.g. smoking) put patients with wounds at greater risk.

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To be continued……..

Highlights of the coming issue


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