

## II. PERALATAN DAN PROSEDUR LABORATORIUM

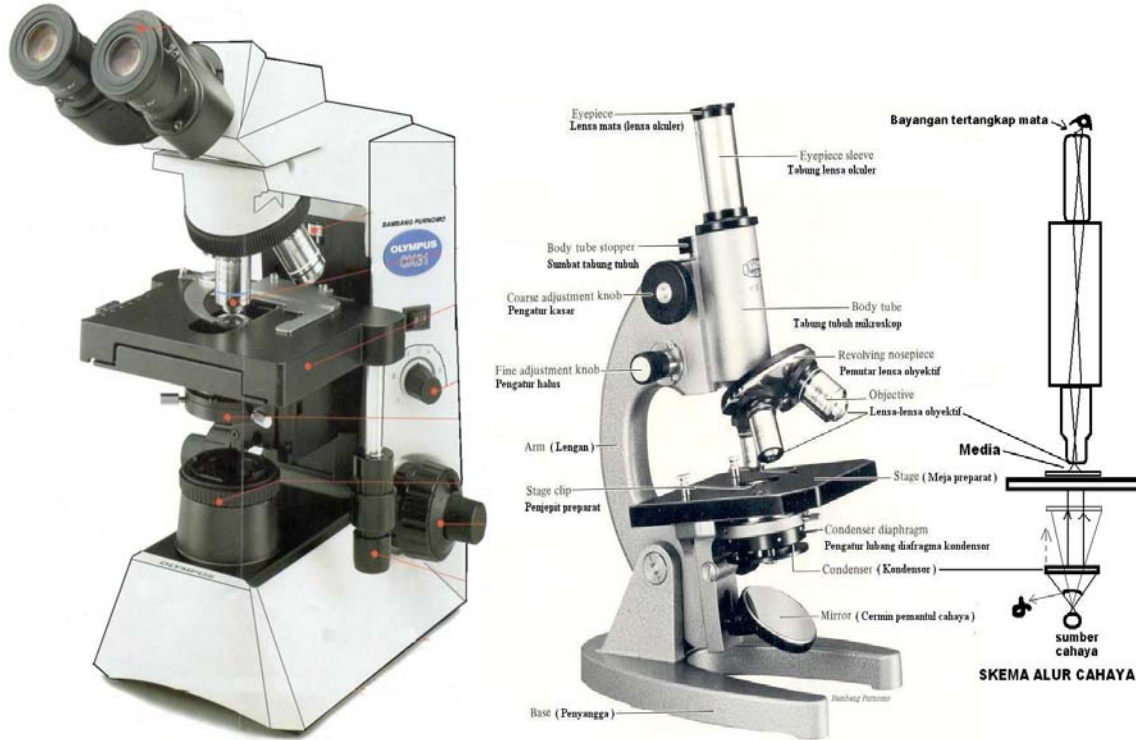
Prosedur dan peralatan tertentu digunakan untuk mempelajari mikroorganisme, misalnya : memelihara, memisahkan (mengisolasi) dalam biakan murni, mengamati karakter dan mengidentifikasi. Telah diketahui bahwa mikroorganisme berada dimana-mana dalam jumlah yang sangat banyak dan beranggotakan banyak spesies. Atas dasar bahwa jumlah dan jenis mikroorganisme yang sangat banyak serta ukurannya yang sangat kecil maka metode dan prosedur laboratorisnya sangat khusus. Oleh karena itu untuk mempelajari spesies atau jenis mikroorganisme tertentu maka langkah pertama adalah memisahkan mikroorganisme yang bersangkutan dari jenis mikroorganisme yang lain. Disamping itu, karena ukuran mikroorganisme sangat kecil maka tidak terlihat jelas oleh mata, sehingga untuk mengamatinya diperlukan alat pembesar, berupa mikroskop. Prosedur laboratoris harus dikembangkan atau diarahkan supaya mampu memisahkan setiap mikroorganisme untuk dikulturkan (ditumbuhkan) secara terpisah. Kumpulan atau massa mikroorganisme dari spesies sama yang dikulturkan secara terpisah dan bebas dari mikroorganisme lain disebut sebagai biakan murni. Massa mikroorganisme dari satu spesies yang sama disebut koloni. Oleh karena itu biakan murni idealnya hanya mengandung satu koloni saja.

Biakan murni yang pertama, diperoleh J. Lister (1879) menggunakan metode seri pengenceran dalam medium cair, namun demikian biakan murni dalam medium cair sulit membentuk koloni yang terpisah sehingga akhirnya dikembangkan medium padat. Oleh karena itu, biakan murni harus dikembangkan dalam mikrobiologi karena sangat bermanfaat di segala bidang kegiatan mikrobiologi, baik untuk pengamatan karakter, penelitian sampai pemanfaatan keunggulannya atau mikrobiologi terapan.

### A. MIKROSKOP DAN MIKROSKOPI

Mikrobiologi dapat dianggap dimulai sejak manusia dapat membuat alat pembesar yang cukup mampu melihat benda yang sangat kecil. Meskipun barangkali Antony van Leeuwenhoek (1632-1723) bukan orang pertama yang melihat bakteri dan protozoa, tetapi dialah yang melaporkan pertama kali melihatnya, kemudian menggambar dan mendiskripsikan mikroorganisme. Alat pembesar yang digunakan Leeuwenhoek merupakan mikroskop pertama dengan menggunakan lensa tunggal. Mikroskop sekarang merupakan mikroskop menggunakan lensa majemuk. Mikroskop merupakan salah satu alat yang erat

sekali hubungannya dengan mikrobiologi, khususnya untuk melihat bayangan mikroorganisme dan bagian-bagiannya yang ukurannya sangat kecil. Berikut ini contoh gambar mikroskop cahaya elektrik (kiri) dan mikroskop cahaya pantul cermin (kanan)



**Mikroskop** (bahasa Yunani: *micron* = kecil dan *scopos* = tujuan) adalah sebuah alat untuk melihat obyek yang terlalu kecil untuk dilihat dengan mata telanjang. Ilmu yang mempelajari benda kecil dengan menggunakan alat ini disebut mikroskopi, dan kata **mikroskopik** berarti sangat kecil, tidak mudah terlihat oleh mata.

### Jenis-jenis mikroskop

Jenis paling umum dari mikroskop, dan yang pertama diciptakan, adalah *mikroskop optis*. Mikroskop ini merupakan alat optik yang terdiri dari satu atau lebih lensa yang memproduksi gambar yang diperbesar dari sebuah benda yang diletakkan di bidang fokal dari lensa tersebut.

Berdasarkan sumber cahayanya, mikroskop dibagi menjadi dua, yaitu, mikroskop cahaya dan mikroskop elektron. Mikroskop cahaya sendiri dibagi lagi menjadi dua kelompok besar, yaitu berdasarkan kegiatan pengamatan dan kerumitan kegiatan pengamatan yang dilakukan. Berdasarkan kegiatan pengamatannya, mikroskop cahaya dibedakan menjadi mikroskop diseksi untuk mengamati bagian permukaan dan mikroskop monokuler dan binokuler untuk mengamati bagian dalam sel. Mikroskop monokuler merupakan mikroskop yang hanya memiliki 1 lensa okuler dan binokuler memiliki 2 lensa okuler. Berdasarkan kerumitan kegiatan pengamatan yang dilakukan, mikroskop dibagi menjadi 2 bagian, yaitu mikroskop sederhana (yang umumnya digunakan pelajar) dan mikroskop riset (mikroskop *dark-field*, fluoresens, fase kontras, *Nomarski DIC*, dan konfokal).

### **Struktur mikroskop**

Tubuh mikroskop pada dasarnya terdiri dari dua bagian utama, yaitu: bagian optik dan bagian non-optik (mekanik). Beberapa jenis mikroskop juga dilengkapi bagian elektrik, fotografi dan scanning. Bagian mekanik dan bagian optik selalu ada pada setiap jenis mikroskop, meskipun tidak semua sub-bagian ada. Bagian mekanik meliputi : statif, tubus, revolver, meja benda, pengatur tubus, pengatur kondensor, diafragma, pengatur meja benda, pengatur atau penjepit preparat dan sumber cahaya. Bagian optiknya meliputi: lensa okuler, lensa obyektif, lensa kondensor, lensa filter dan cermin pengatur cahaya.

### **Pembesaran**

Mikroskop merupakan alat yang dapat menghasilkan bayangan dari benda yang di mikroskop menjadi lebih besar. Pembesaran ini tergantung pada berbagai faktor, diantaranya titik fokus kedua lensa( objektif  $f_1$  dan okuler  $f_2$ , panjang tubulus atau jarak(t) lensa objektif terhadap lensa okuler dan yang ketiga adalah jarak pandang mata normal(sn). Rumus: 
$$V_m = \frac{t \cdot sn}{f_1 \cdot f_2}$$

Bayangan benda (obyek) yang kita lihat dibentuk dan diperbesar oleh lensa obyektif, di dalam tubus mikroskop membentuk bayangan nyata terbalik dari obyek. Bayangan nyata tersebut selanjutnya dibalik dan diperbesar lagi oleh lensa okuler. Lensa okuler merupakan lensa yang

berfungsi untuk membuat bayangan terakhir, sehingga bayangan tersebut dapat dilihat langsung oleh mata pengamat.

Lensa yang baik diperoleh dengan memperhatikan pembesaran dan daya pisahnya. Semakin pendek jarak titik api lensa akan semakin kuat pembesarannya, sehingga semakin besar kemampuan suatu lensa akan semakin kecil jarak dua titik api yang berdekatan yang dapat dilihat secara terpisah menggunakan mikroskop.

Beberapa lensa obyektif biasanya dipasang pada roda berputar yang disebut revolver. Setiap lensa obyektif dapat diputar ke tempat yang sesuai dengan pembesaran yang diinginkan. Lensa obyektif dibuat dalam beberapa pembesaran yang berbeda, yakni : 4x, 10x, 40x, dan 100x, demikian juga lensa okuler tersedia beberapa pembesaran, yakni : 4x, 10x, 16x, dan 20x. Lensa okuler dipasang pada ujung dalam tabung dan biasanya yang dipasang adalah yang pembesaran 10x. Dengan demikian jika kita mengamati objek menggunakan lensa okuler pembesaran 10x dan lensa obyektif 40x, maka pembesaran objek yang kita lihat menjadi 400x dibanding besarnya objek yang sebenarnya.

Kondensor berfungsi sebagai pengatur intensitas cahaya yang masuk ke dalam mikroskop. Kondensor mempunyai dua bagian penting, yaitu :

1. Susunan lensa untuk mengumpulkan sinar sebelum masuk ke dalam objek dan lensa obyektif.
2. Diafragma berfungsi untuk mengatur sinar tepi yang masuk ke dalam lensa obyektif dan okuler.

Hukum fisika menyatakan bahwa bagian terkecil yang dapat kita lihat disebut daya pisah, yaitu : kemampuan lensa untuk menghasilkan bayangan berlainan dari dua titik yang berdekatan. Daya pisah dibatasi oleh panjang gelombang cahaya yang digunakan untuk menerangi preparat dan jarak titik api lensa-lensa dalam mikroskop. Batas daya pisah pada cahaya biasa yang dapat dilihat  $\pm 0,2 \mu\text{m}$ , sehingga dua benda yang jaraknya kurang dari  $0,2 \mu\text{m}$  akan terlihat sebagai satu benda, begitu juga benda yang ukurannya kurang dari  $0,2 \mu\text{m}$  tidak akan terlihat jika menggunakan sumber cahaya biasa. Oleh karena itu untuk melihat

benda yang ukurannya kurang dari  $0,2 \mu\text{m}$  atau melihat dua benda yang jaraknya kurang dari  $0,2 \mu\text{m}$  diperlukan sumber cahaya lain yang mempunyai panjang gelombang lebih pendek.

Daya pisah suatu mikroskop cahaya ditentukan oleh panjang gelombang cahaya dan sifat lensa (*numerical aperture* = NA) yang dirumuskan  $d = \lambda/NA$  dengan arti lambang :  $d$  = daya pisah  $\lambda$  = panjang gelombang, dan NA = *numerical aperture* atau angka singkapan. Dari rumus tersebut diketahui bahwa daya pisah dapat ditingkatkan dengan cara memperkecil panjang gelombang dan memperbesar angka singkapan lensa. Panjang gelombang cahaya yang digunakan dalam mikroskop cahaya terbatas pada gelombang cahaya kasat mata yang berkisar dari 400 nm sampai 700 nm dan angka singkapan 0,85 pada medium udara dan 1,2 sampai 1,4 pada medium celup cair.

Untuk menggambarkan daya pisah, misalnya panjang gelombang cahaya biasa yang digunakan dan mengamati preparat kering maka daya pisahnya =  $700 \text{ nm} / 0,85 = 824 \text{ nm}$  sedangkan jika kita menggunakan filter lensa hijau panjang gelombang terpilih 550 nm, maka daya pisahnya menjadi =  $550 \text{ nm} / 0,85 = 467 \text{ nm}$  dan jika menggunakan preparat celup minyak NA lensa menjadi sebesar 1,25 dan NA kondensor sebesar 0,9, sehingga daya pisahnya menjadi  $550 \text{ nm} / (1,25+0,9) = 225 \text{ nm}$ . Dengan demikian benda-benda dalam preparat kering yang diamati menggunakan mikroskop cahaya akan terlihat jika ukurannya lebih dari  $0,55 \mu\text{m}$  dan terlihat terpisah jika jaraknya lebih dari  $0,55 \mu\text{m}$ , sedangkan dengan memilih cahaya hijau ukuran benda yang terlihat dan jarak yang terpisah menjadi  $0,467 \mu\text{m}$  dan jika menggunakan preparat celup minyak daya pisahnya menjadi lebih besar lagi atau ukuran benda yang terlihat dan jarak yang terpisah menjadi lebih kecil, yaitu  $0,225 \mu\text{m}$ .

### **Sifat bayangan**

baik lensa objektif maupun lensa okuler keduanya merupakan lensa cembung Secara sederhana dan garis besar lensa objektif menghasilkan suatu bayangan sementara yang mempunyai sifat semu, terbalik, dan diperbesar terhadap posisi benda mula mula. baik pada mikroskop cahaya maupun mikroskop elektron. Yang menentukan sifat bayangan akhir selanjutnya adalah lensa okuler. Pada mikroskop cahaya bayangan akhir mempunyai sifat yang sama seperti bayangan sementara semu, terbalik, dan lebih lagi diperbesar. Pada mikroskop elektron bayangan akhir

mempunyai sifat yang sama seperti gambar benda nyata, sejajar, dan diperbesar. Petunjuk: Jika seseorang menggunakan mikroskop cahaya dia meletakkan huruf A dibawah mikroskop maka yang dia lihat pada mikroskop tampilan bayangan tersebut adalah huruf tersebut hanya terbalik dan diperbesar.

## **Mikroskop cahaya**

Mikroskop cahaya atau dikenal juga dengan nama "*Compound light microscope*" adalah sebuah mikroskop yang menggunakan cahaya lampu sebagai pengganti cahaya matahari sebagaimana yang digunakan pada mikroskop konvensional. Pada mikroskop konvensional, sumber cahaya masih berasal dari sinar matahari yang dipantulkan dengan suatu cermin datar ataupun cekung yang terdapat dibawah kondensor. Cermin ini akan mengarahkan cahaya dari luar kedalam kondensor.

## **Jenis lensa**

Mikroskop cahaya menggunakan tiga jenis lensa, yaitu lensa obyektif, lensa okuler, dan kondensor.

Lensa obyektif dan lensa okuler terletak pada kedua ujung tabung mikroskop sedangkan penggunaan lensa okuler terletak pada mikroskop bisa berbentuk lensa tunggal (monokuler) atau ganda (binokuler). Pada ujung bawah mikroskop terdapat tempat dudukan lensa obyektif yang bisa dipasang tiga lensa atau lebih. Di bawah tabung mikroskop terdapat meja mikroskop yang merupakan tempat preparat.

Sistem lensa yang ketiga adalah kondensor. Kondensor berperan untuk menerangi obyek dan lensa-lensa mikroskop yang lain.

## **Cara kerja**

- **Lensa obyektif** berfungsi guna pembentukan bayangan pertama dan menentukan struktur serta bagian renik yang akan terlihat pada bayangan akhir serta berkemampuan untuk memperbesar bayangan obyek sehingga dapat memiliki nilai "apertura" yaitu suatu ukuran daya pisah suatu lensa obyektif yang akan menentukan daya pisah spesimen,

sehingga mampu menunjukkan struktur renik yang berdekatan sebagai dua benda yang terpisah.

- **Lensa okuler**, adalah lensa mikroskop yang terdapat di bagian ujung atas tabung berdekatan dengan mata pengamat, dan berfungsi untuk memperbesar bayangan yang dihasilkan oleh lensa obyektif berkisar antara 4 hingga 25 kali.
- **Lensa kondensor**, adalah lensa yang berfungsi guna mendukung terciptanya pencahayaan pada obyek yang akan dilihat sehingga dengan pengaturan yang tepat maka akan diperoleh daya pisah maksimal.

Jika daya pisah kurang maksimal maka dua benda akan terlihat menjadi satu dan pembesarannya pun akan kurang optimal.

### **Preparasi sediaan**

Persiapan preparat di dalam mikroskop cahaya terbagi menjadi dua jenis, yaitu :

- **Preparat Non-permanen**, yang dapat diperoleh dengan menambahkan air pada sel hidup di atas kaca objek, kemudian diamati di bawah mikroskop.
- **Preparat permanen**, yang dapat diperoleh dengan melakukan fiksasi yang bertujuan untuk membuat sel dapat menyerap warna, membuat sel tidak bergerak, mematikan sel, dan mengawetkannya.
- **Tahap selanjutnya**, yaitu pembuatan sayatan, yang bertujuan untuk memotong sayatan hingga setipis mungkin agar mudah diamati di bawah mikroskop. preparat dilapisi dengan monomer resin melalui proses pemanasan karena pada umumnya jaringan memiliki tekstur yang lunak dan mudah pecah setelah mengalami fiksasi, kemudian dilanjutkan dengan pemotongan menggunakan mikrotom. Umumnya mata pisau mikrotom terbuat dari berlian karena berlian tersusun dari atom karbon yang padat. Oleh karena itu, sayatan yang terbentuk lebih rapi. Setelah dilakukan penyayatan, dilanjutkan dengan pewarnaan, yang bertujuan untuk memperbesar kontras antara preparat yang akan diamati dengan lingkungan sekitarnya. Setiap pewarna mengikat molekul yang memiliki kespesifikan tertentu, contohnya : Hematoksilin, yang mampu mengikat asam amino basa

(lisin dan arginin) pada berbagai protein, dan eosin, yang mampu mengikat molekul asam (DNA dan rantai samping pada aspartat dan glutamat).

## **Mikroskop elektron**

**Mikroskop elektron** adalah sebuah mikroskop yang mampu untuk melakukan pembesaran objek sampai 2 juta kali, yang menggunakan elektro statik dan elektro magnetik untuk mengontrol pencahayaan dan tampilan gambar serta memiliki kemampuan pembesaran objek serta resolusi yang jauh lebih bagus daripada mikroskop cahaya. Mikroskop elektron ini menggunakan jauh lebih banyak energi dan radiasi elektromagnetik yang lebih pendek dibandingkan mikroskop cahaya.

## **Fenomena elektron**

Pada tahun 1920 ditemukan suatu fenomena di mana elektron yang dipercepat dalam suatu kolom elektromagnet, dalam suasana hampa udara (vakum) berkarakter seperti cahaya, dengan panjang gelombang yang 100.000 kali lebih kecil dari cahaya. Selanjutnya ditemukan juga bahwa medan listrik dan medan magnet dapat berperan sebagai lensa dan cermin seperti pada lensa gelas dalam mikroskop cahaya.

## **Jenis-jenis mikroskop elektron**

### **Mikroskop transmisi elektron (TEM)**

Mikroskop transmisi elektron (Transmission electron microscope-TEM) adalah sebuah mikroskop elektron yang cara kerjanya mirip dengan cara kerja proyektor slide, di mana elektron ditembuskan ke dalam obyek pengamatan dan pengamat mengamati hasil tembusannya pada layar.

### **Sejarah penemuan TEM**

Seorang ilmuwan dari universitas Berlin yaitu Dr. Ernst Ruska <sup>[1]</sup> menggabungkan penemuan ini dan membangun mikroskop transmisi elektron (TEM) yang pertama pada tahun 1931. Untuk hasil karyanya ini maka dunia ilmu pengetahuan menganugerahinya hadiah Penghargaan Nobel



dalam fisika pada tahun 1986. Mikroskop yang pertama kali diciptakannya adalah dengan menggunakan dua lensa medan magnet, namun tiga tahun kemudian ia menyempurnakan karyanya tersebut dengan menambahkan lensa ketiga dan mendemonstrasikan kinerjanya yang menghasilkan resolusi hingga 100 nanometer (nm) (dua kali lebih baik dari mikroskop cahaya pada masa itu).

### **Cara kerja**

Mikroskop transmisi elektron saat ini telah mengalami peningkatan kinerja hingga mampu menghasilkan resolusi hingga 0,1 nm (atau 1 angstrom) atau sama dengan pembesaran sampai satu juta kali. Meskipun banyak bidang-bidang ilmu pengetahuan yang berkembang pesat dengan bantuan mikroskop transmisi elektron ini.

Adanya persyaratan bahwa "obyek pengamatan harus setipis mungkin" ini kembali membuat sebagian peneliti tidak terpuaskan, terutama yang memiliki obyek yang tidak dapat dengan serta merta dipertipis. Karena itu pengembangan metode baru mikroskop elektron terus dilakukan.

### **Preparasi sediaan TEM**

Agar pengamat dapat mengamati preparat dengan baik, diperlukan persiapan sediaan dengan tahap sebagai berikut: 1. melakukan fiksasi, yang bertujuan untuk mematkan sel tanpa mengubah struktur sel yang akan diamati. fiksasi dapat dilakukan dengan menggunakan senyawa glutaraldehid atau osmium tetroksida. 2. pembuatan sayatan, yang bertujuan untuk memotong sayatan hingga setipis mungkin agar mudah diamati di bawah mikroskop. Preparat dilapisi dengan monomer resin melalui proses pemanasan, kemudian dilanjutkan dengan pemotongan menggunakan mikrotom. Umumnya mata pisau mikrotom terbuat dari berlian karena berlian tersusun dari atom karbon yang padat. Oleh karena itu, sayatan yang terbentuk lebih rapi. Sayatan yang telah terbentuk diletakkan di atas cincin berpetak untuk diamati. 3. pelapisan/pewarnaan, bertujuan untuk memperbesar kontras antara preparat yang akan diamati dengan lingkungan sekitarnya. Pelapisan/pewarnaan dapat menggunakan logam berat seperti uranium dan timbal.

### **Mikroskop pemindai transmisi elektron (STEM)**

Mikroskop pemindai transmisi elektron (STEM) adalah merupakan salah satu tipe yang merupakan hasil pengembangan dari mikroskop transmisi elektron (TEM).

Pada sistem STEM ini, electron menembus spesimen namun sebagaimana halnya dengan cara kerja SEM, optik elektron terfokus langsung pada sudut yang sempit dengan memindai obyek menggunakan pola pemindaian dimana obyek tersebut dipindai dari satu sisi ke sisi lainnya (*raster*) yang menghasilkan lajur-lajur titik (*dots*) yang membentuk gambar seperti yang dihasilkan oleh CRT pada televisi / monitor.

### **Mikroskop pemindai elektron (SEM)**

Mikroskop pemindai elektron (SEM) yang digunakan untuk studi detil arsitektur permukaan sel (atau struktur jasad renik lainnya), dan obyek diamati secara tiga dimensi.

### **Sejarah penemuan SEM**

Tidak diketahui secara persis siapa sebenarnya penemu Mikroskop pemindai elektron (Scanning Electron Microscope-SEM) ini. Publikasi pertama kali yang mendiskripsikan teori SEM dilakukan oleh fisikawan Jerman dr. Max Knoll pada 1935, meskipun fisikawan Jerman lainnya Dr. Manfred von Ardenne mengklaim dirinya telah melakukan penelitian suatu fenomena yang kemudian disebut SEM hingga tahun 1937. Mungkin karena itu, tidak satu pun dari keduanya mendapatkan hadiah nobel untuk penemuan itu.

Pada 1942 tiga orang ilmuwan Amerika yaitu Dr. Vladimir Kosma Zworykin<sup>[2]</sup>, Dr. James Hillier, dan Dr. Snijder, benar-benar membangun sebuah mikroskop elektron metode pemindaian (SEM) dengan resolusi hingga 50 nm atau magnifikasi 8.000 kali. Sebagai perbandingan SEM modern sekarang ini mempunyai resolusi hingga 1 nm atau pembesaran 400.000 kali. Mikroskop elektron cara ini memfokuskan sinar elektron (electron beam) di permukaan obyek dan mengambil gambarnya dengan mendeteksi elektron yang muncul dari permukaan obyek.

### **Cara kerja SEM**

Cara terbentuknya gambar pada SEM berbeda dengan apa yang terjadi pada mikroskop optic dan TEM. Pada SEM, gambar dibuat berdasarkan deteksi elektron baru (elektron sekunder) atau

elektron pantul yang muncul dari permukaan sampel ketika permukaan sampel tersebut dipindai dengan sinar elektron. Elektron sekunder atau elektron pantul yang terdeteksi selanjutnya diperkuat sinyalnya, kemudian besar amplitudonya ditampilkan dalam gradasi gelap-terang pada layar monitor CRT (cathode ray tube). Di layar CRT inilah gambar struktur obyek yang sudah diperbesar bisa dilihat. Pada proses operasinya, SEM tidak memerlukan sampel yang ditipiskan, sehingga bisa digunakan untuk melihat obyek dari sudut pandang 3 dimensi.

### **Preparasi sediaan SEM**

Agar pengamat dapat mengamati preparat dengan baik, diperlukan persiapan sediaan dengan tahap sebagai berikut: 1. melakukan fiksasi, yang bertujuan untuk mematikan sel tanpa mengubah struktur sel yang akan diamati. fiksasi dapat dilakukan dengan menggunakan senyawa glutaraldehida atau osmium tetroksida. 2. dehidrasi, yang bertujuan untuk memperendah kadar air dalam sayatan sehingga tidak mengganggu proses pengamatan. 3. pelapisan/pewarnaan, bertujuan untuk memperbesar kontras antara preparat yang akan diamati dengan lingkungan sekitarnya. Pelapisan/pewarnaan dapat menggunakan logam mulia seperti emas dan platina.

### **Mikroskop pemindai lingkungan elektron (ESEM)**

Mikroskop ini adalah merupakan pengembangan dari SEM, yang dalam bahasa Inggrisnya disebut *Environmental SEM* (ESEM) yang dikembangkan guna mengatasi obyek pengamatan yang tidak memenuhi syarat sebagai obyek TEM maupun SEM.

Obyek yang tidak memenuhi syarat seperti ini biasanya adalah bahan alami yang ingin diamati secara detail tanpa merusak atau menambah perlakuan yang tidak perlu terhadap obyek yang apabila menggunakan alat SEM konvensional perlu ditambahkan beberapa trik yang memungkinkan hal tersebut bisa terlaksana.

### **Sejarah penemuan**

Teknologi ESEM ini dirintis oleh Gerasimos D. Danilatos, seorang kelahiran Yunani yang bermigrasi ke Australia pada akhir tahun 1972 dan memperoleh gelar Ph.D dari Universitas New

South Wales (UNSW) pada tahun 1977 dengan judul disertasi *Dynamic Mechanical Properties of Keratin Fibres* .

Dr. Danilatos ini dikenal sebagai pionir dari teknologi ESEM, yang merupakan suatu inovasi besar bagi dunia mikroskop elektron serta merupakan kemajuan fundamental dari ilmu mikroskopi.

Deengan teknologi ESEM ini maka dimungkinkan bagi seorang peneliti untuk meneliti sebuah objek yang berada pada lingkungan yang menyerupai gas yang betekanan rendah (*low-pressure gaseous environments*) misalnya pada 10-50 Torr serta tingkat humiditas diatas 100%. Dalam arti kata lain ESEM ini memungkinkan dilakukannya penelitian obyek baik dalam keadaan kering maupun basah.

Sebuah perusahaan di Boston yaitu Electro Scan Corporation pada tahun 1988 ( perusahaan ini diambil alih oleh Philips pada tahun 1996- sekarang bernama FEI Company <sup>[3]</sup> telah menemukan suatu cara guna menangkap elektron dari obyek untuk mendapatkan gambar dan memproduksi muatan positif dengan cara mendesain sebuah detektor yang dapat menangkap elektron dari suatu obyek dalam suasana tidak vakum sekaligus menjadi produsen ion positif yang akan dihantarkan oleh gas dalam ruang obyek ke permukaan obyek. Beberapa jenis gas telah dicoba untuk menguji teori ini, di antaranya adalah beberapa gas ideal, gas , dan lain lain. Namun, yang memberikan hasil gambar yang terbaik hanyalah uap air. Untuk sample dengan karakteristik tertentu uap air kadang kurang memberikan hasil yang maksimum.

Pada beberapa tahun terakhir ini peralatan ESEM mulai dipasarkan oleh para produsennya dengan mengiklankan gambar-gambar jasad renik dalam keadaan hidup yang selama ini tidak dapat terlihat dengan mikroskop elektron.

### **Cara kerja**

Pertama-tama dilakukan suatu upaya untuk menghilangkan penumpukan elektron (*charging*) di permukaan obyek, dengan membuat suasana dalam ruang sample tidak vakum tetapi diisi dengan sedikit gas yang akan mengantarkan muatan positif ke permukaan obyek, sehingga penumpukan elektron dapat dihindari.

Hal ini menimbulkan masalah karena kolom tempat elektron dipercepat dan ruang filamen di mana elektron yang dihasilkan memerlukan tingkat vakum yang tinggi. Permasalahan ini dapat diselesaikan dengan memisahkan sistem pompa vakum ruang obyek dan ruang kolom serta filamen, dengan menggunakan sistem pompa untuk masing-masing ruang. Di antaranya kemudian dipasang satu atau lebih piringan logam platina yang biasa disebut (aperture) berlubang dengan diameter antara 200 hingga 500 mikrometer yang digunakan hanya untuk melewatkan elektron, sementara tingkat kevakuman yang berbeda dari tiap ruangan tetap terjaga.

### **Tipe-tipe pengembangan**

#### **Mikroskop refleksi elektron (REM)**

Yang dalam bahasa Inggrisnya disebut Reflection electron microscope (REM), adalah mikroskop elektron yang memiliki cara kerja yang serupa sebagaimana halnya dengan cara kerja TEM namun sistem ini menggunakan deteksi pantulan elektron pada permukaan objek. Teknik ini secara khusus digunakan dengan menggabungkannya dengan teknik Refleksi difraksi elektron energi tinggi (*Reflection High Energy Electron Diffraction*) dan teknik Refleksi pelepasan spektrum energi tinggi (*reflection high-energy loss spectrum - RHELS*)

#### ***Spin-Polarized Low-Energy Electron Microscopy (SPLEEM)***

*Spin-Polarized Low-Energy Electron Microscopy (SPLEEM)* ini adalah merupakan Variasi lain yang dikembangkan dari teknik yang sudah ada sebelumnya, yang digunakan untuk melihat struktur mikro dari medan magnet (en:magnetic domains).

#### **Teknik pembuatan preparat yang digunakan pada mikroskop elektron**

Materi yang akan dijadikan objek pemantauan dengan menggunakan mikroskop elektron ini harus diproses sedemikian rupa sehingga menghasilkan suatu sampel yang memenuhi syarat untuk dapat digunakan sebagai preparat pada mikroskop elektron.

Teknik yang digunakan dalam pembuatan preparat ada berbagai macam tergantung pada spesimen dan penelitian yang dibutuhkan, antara lain :

- Kriofiksasi yaitu suatu metode persiapan dengan menggunakan teknik pembekuan spesimen dengan cepat yang menggunakan nitrogen cair ataupun helium cair, dimana air yang ada akan membentuk kristal-kristal yang menyerupai kaca. Suatu bidang ilmu yang disebut mikroskopi cryo-elektron (*cryo-electron microscopy*) telah dikembangkan berdasarkan tehnik ini. Dengan pengembangan dari Mikroskopi cryo-elektron dari potongan menyerupai kaca (vitreous) atau disebut *cryo-electron microscopy of vitreous sections (CEMOVIS)*, maka sekarang telah dimungkinkan untuk melakukan penelitian secara virtual terhadap specimen biologi dalam keadaan aslinya.
- Fiksasi - yaitu suatu metode persiapan untuk menyiapkan suatu sampel agar tampak realistik (seperti kenyataannya ) dengan menggunakan glutaraldehid dan osmium tetroksida.
- Dehidrasi - yaitu suatu metode persiapan dengan cara menggantikan air dengan bahan pelarut organik seperti misalnya ethanol atau acetone.
- Penanaman (*Embedding*) - yaitu suatu metode persiapan dengan cara menginfiltrasi jaringan dengan resin seperti misalnya araldit atau epoksi untuk pemisahan bagian.
- Pembelahan (*Sectioning*)- yaitu suatu metode persiapan untuk mendapatkan potongan tipis dari spesimen sehingga menjadikannya semi transparan terhadap elektron. Pemotongan ini bisa dilakukan dengan ultramicrotome dengan menggunakan pisau berlian untuk menghasilkan potongan yang tipis sekali. Pisau kaca juga biasa digunakan oleh karena harganya lebih murah.
- Pewarnaan (*Staining*) - yaitu suatu metode persiapan dengan menggunakan metal berat seperti timah, uranium, atau tungsten untuk menguraikan elektron gambar sehingga menghasilkan kontras antara struktur yang berlainan di mana khususnya materi biologikal banyak yang warnanya nyaris transparan terhadap elektron (objek fase lemah).
- Pembekuan fraktur (*Freeze-fracture*) - yaitu suatu metode persiapan yang biasanya digunakan untuk menguji membran lipid. Jaringan atau sel segar didinginkan dengan cepat (*cryofixed*) kemudian dipatah-patahkan atau dengan menggunakan *microtome* sewaktu masih berada dalam keadaan suhu nitrogen ( hingga mencapai -100% Celsius).

Patahan beku tersebut lalu diuapi dengan uap platinum atau emas dengan sudut 45 derajat pada sebuah alat evaporator en:evaporator tekanan tinggi.

- *Ion Beam Milling* - yaitu suatu metode mempersiapkan sebuah sampel hingga menjadi transparan terhadap elektron dengan menggunakan cara pembakaran ion( biasanya digunakan argon) pada permukaan dari suatu sudut hingga memercikkan material dari permukaannya. Kategori yang lebih rendah dari metode *Ion Beam Milling* ini adalah metode berikutnya adalah metode *Focused ion beam milling*, dimana galium ion digunakan untuk menghasilkan selaput elektron transparan pada suatu bagian spesifik pada sampel.
- Pelapisan konduktif (*Conductive Coating*) - yaitu suatu metode mempersiapkan lapisan ultra tipis dari suatu material *electrically-conducting* . Ini dilakukan untuk mencegah terjadinya akumulasi dari medan elektrik statis pada spesimen sehubungan dengan elektron iradiasi sewaktu proses penggambaran sampel. Beberapa bahan pelapis termasuk emas, palladium (emas putih), platinum, tungsten, graphite dan lain-lain, secara khusus sangatlah penting bagi penelitian spesimen dengan SEM.

### **Pembuatan film dengan mikroskop ESEM**

Dengan melakukan penambahan peralatan video maka pengamat dapat melakukan pengamatan secara terus menerus pada obyek yang hidup.

Sebuah perusahaan film dari Perancis bahkan berhasil merekam kehidupan makhluk kecil dan memfilmkannya secara nyata. Dari beberapa film yang dibuat, film berjudul *Cannibal Mites*<sup>[4]</sup> memenangkan beberapa penghargaan di antaranya *Edutainment award* (Jepang 1999), *Best scientific photography award* (Perancis 1999), dan *Grand prix-best popular and informative scientific film* (Perancis 1999). Film ini ditayangkan juga di stasiun televisi *Zweites Deutsches Fernsehen* (en:ZDF) Jerman, Discovery Channel di AS dan Britania Raya. Kini perusahaan yang sama tengah menggarap film seri berjudul "*Fly Wars*"<sup>[5]</sup> yang rata-rata memakai sekitar lima menit pengambilan gambar dengan ESEM, pada film tersebut dapat dilihat dengan detail setiap lembar bulu yang dimiliki lalat dalam pertempurannya.

## B. PERALATAN DARI GELAS



◀ Botol gelas coklat dengan beberapa peralatan gelas laboratorium di belakangnya

**Peralatan gelas laboratorium** merujuk pada berbagai peralatan laboratorium yang terbuat dari glas, yang digunakan dalam percobaan ilmiah, terutama dalam laboratorium kimia dan biologi. Beberapa peralatan tersebut sekarang ada yang telah dibuat dari plastik, namun peralatan gelas masih sering digunakan oleh karena sifat gelas yang inert, transparan, dan tahan panas.

Gelas borosilikat, dahulu dinamakan Pyrex, sering digunakan karena sifatnya yang tahan dengan tegangan termal. Untuk beberapa aplikasi, kuarsa digunakan oleh karena ia tahan panas dalam temperatur yang tinggi dan memiliki sifat terawang di beberapa spektrum elektromagnetis. Di beberapa aplikasi, terutama pada botol penyimpanan, gelas berwarna coklat tua biasanya digunakan untuk menghindarkan zat yang disimpan dari cahaya luar. Peralatan yang terbuat dari material lainnya juga digunakan untuk tujuan tertentu, misalnya asam hidroklorida yang disimpan dalam polietilena karena asam ini dapat melarutkan gelas.

### Gelas Beker



◀ Beker dalam berbagai ukuran volume (kanan)

**Gelas beker** atau lebih sering disebut ‘beker’ saja adalah sebuah wadah penampung yang digunakan untuk mengaduk, mencampur, dan memanaskan cairan yang biasanya digunakan dalam laboratorium. Beker secara umum berbentuk silinder dengan dasar yang bidang dan tersedia dalam berbagai ukuran, mulai dari beberapa mL sampai beberapa liter.



Beker dapat terbuat dari gelas (umumnya glas borosilikat ataupun dari plastik. Beker yang digunakan untuk menampung zat kimia yang korosif seperti asam atau zat-zat lainnya yang sangat reaktif biasanya terbuat dari PTFE ataupun bahan-bahan yang reaktivitasnya rendah. Beker dapat ditutup dengan glas pengamat untuk mencegah kontaminasi dan penyusutan zat. Beker seringkali dibubuhi dengan ukuran yang terdapat pada sisi beker yang mengindikasikan volume tertampung. Sebagai contoh, beker dengan volume 250 mL ditandai dengan garis-garis yang mengindikasikan volume zat tertampung sebesar 50, 100, 150, 200, dan 250 mL. Keakuratan ukuran ini sangat bervariasi. Beker berbeda dengan labu laboratorium terlihat dari sisinya yang lurus dan bukannya miring. Biasanya beker lebih sering digunakan dalam percobaan kimia dasar.

## Buret

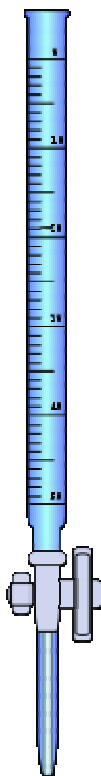


Diagram buret  
modern

**Buret** adalah sebuah peralatan gelas laboratorium berbentuk silinder yang memiliki garis ukur dan sumbat keran pada bagian bawahnya. Ia digunakan untuk meneteskan sejumlah reagen cair dalam eksperimen yang memerlukan presisi, seperti pada eksperimen titrasi. Buret sangatlah akurat, buret kelas A memiliki akurasi sampai dengan  $\pm 0,05 \text{ cm}^3$ .

Oleh karena presisi buret yang tinggi, kehati-hatian pengukuran volume dengan buret sangatlah penting untuk menghindari galat sistematik. Ketika membaca buret, mata harus tegak lurus dengan permukaan cairan untuk menghindari galat paralaks. Bahkan ketebalan garis ukur juga mempengaruhi; bagian bawah meniskus cairan harus menyentuh bagian atas garis. Kaidah yang umumnya digunakan adalah dengan menambahkan 0,02 mL jika bagian bawah meniskus menyentuh bagian bawah garis ukur. Oleh karena presisinya yang tinggi, satu tetes cairan yang menggantung pada ujung buret harus ditransfer ke labu penerima, biasanya dengan menyentuh tetesan itu ke sisi labu dan membilasnya ke dalam larutan dengan pelarut.

## Cawan Petri



Cawan Petri gelas pireks.

**Cawan Petri** atau **telepa Petri** adalah sebuah wadah yang bentuknya bundar dan terbuat dari plastik atau gelas yang digunakan untuk membiakkan sel. Cawan Petri selalu berpasangan, yang ukurannya agak kecil sebagai wadah dan yang lebih besar merupakan tutupnya. Cawan Petri dinamai menurut nama penemunya pada tahun 1877, yaitu Julius Richard Petri (1852–1921), ahli bakteri berkebangsaan Jerman.

Alat ini digunakan sebagai wadah untuk penyelidikan tropi dan juga untuk mengkultur bakteri, khamir, spora, atau biji-bijian. Cawan Petri plastik dapat dimusnahkan setelah sekali pakai untuk kultur bakteri.

## Corong Büchner



◀ Sebuah corong Büchner yang dihubungkan dengan labu yang terhubung dengan pompa vakum, ditemukan oleh Ernst Büchner

**Corong Büchner** adalah sebuah peralatan laboratorium yang digunakan dalam penyaringan vakum.<sup>[1]</sup> Ia biasanya terbuat dari porselein, namun kadangkala ada juga yang terbuat dari gelas dan plastik. Di bagian atasnya terdapat sebuah silinder dengan dasar yang berpori-pori. **Corong Hirsch** juga memiliki struktur dan kegunaan yang sama, namun ia lebih kecil dan biasanya terbuat dari gelas.

Bahan penyaring (biasanya kertas saring) diletakkan di atas corong tersebut dan dibasahi dengan pelarut untuk mencegah kebocoran pada awal penyaringan. Cairan yang akan disaring ditumpahkan ke dalam corong dan dihisap ke dalam labu dari dasar corong yang berpori dengan pompa vakum.

### Corong pemisah



Corong pemisah. Lapisan eter dengan zat terlarut yang berwarna kuning di bagian atas dan lapisan air di bawahnya.

**Corong pemisah** atau **corong pisah** adalah peralatan laboratorium yang digunakan dalam ekstraksi cair-cair untuk memisahkan komponen-komponen dalam suatu campuran antara dua fase pelarut dengan densitas berbeda yang takcampur.

Umumnya salah satu fase berupa larutan air dan yang lainnya berupa pelarut organik lipofilik seperti eter, MTBE, diklorometana, kloroform, ataupun etil asetat. Kebanyakan pelarut organik berada di atas fase air kecuali pelarut yang memiliki atom dari unsur halogen.

Corong pemisah berbentuk kerucut yang ditutupi setengah bola. Ia mempunyai penyumbat di atasnya dan keran di bawahnya. Corong pemisah yang digunakan dalam laboratorium terbuat dari gelas borosilikat dan kerannya terbuat dari gelas ataupun Teflon. Ukuran corong pemisah bervariasi antara 50 mL sampai 3 L. Dalam skala industri, corong pemisah bisa berukuran sangat besar dan dipasang sentrifuge.

Untuk memakai corong ini, campuran dan dua fase pelarut dimasukkan ke dalam corong dari atas dengan corong keran ditutup. Corong ini kemudian ditutup dan digoyang dengan kuat untuk membuat dua fase larutan tercampur. Corong ini kemudian dibalik dan keran dibuka untuk melepaskan tekanan uap yang berlebihan. Corong ini kemudian dидiamkan agar

pemisahan antara dua fase berlangsung. Penyumbat dan keran corong kemudian dibuka dan dua fase larutan ini dipisahkan dengan mengontrol keran corong.

## C. STERILISASI

**Sterilization** refers to any process that effectively kills or eliminates transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) from a surface, equipment, article of food or medication, or biological culture medium. Sterilization does not, however, remove prions. Sterilization can be achieved through application of heat, chemicals, irradiation, high pressure or filtration.

### 1. Applications

#### 1.1. Foods

The first application of sterilization was thorough cooking to effect the partial heat sterilization of foods and water. Cultures that practice heat sterilization of food and water have longer life expectancy and lower rates of disability. Canning of foods by heat sterilization was an extension of the same principle. Ingestion of contaminated food and water remains a leading cause of illness and death in the developing world, particularly for children.

Food sterilization is usually considered a harsher form of Pasteurization<sup>[3]</sup>, and is carried out through heating, though other methods are available. Food sterilization is commonly a part of canning and is used in combination with or instead of preservatives, refrigeration, and other ways to preserve food.

#### 1.2. Medicine and surgery

In general, surgical instruments and medications that enter an already sterile part of the body (such as the blood, or beneath the skin) must have a high sterility assurance level. Examples of such instruments include scalpels, hypodermic needles and artificial pacemakers. This is also essential in the manufacture of parenteral pharmaceuticals.

Heat sterilization of medical instruments is known to have been used in Ancient Rome, but it mostly disappeared throughout the Middle Ages resulting in significant increases in disability and death following surgical procedures.

Preparation of injectable medications and intravenous solutions for fluid replacement therapy requires not only a high sterility assurance level, but well-designed containers to prevent entry of adventitious agents after initial sterilization.

## 2. Heat sterilization

### 2.1. Steam sterilization



#### Front-loading autoclaves

A widely-used method for heat sterilization is the autoclave. Autoclaves commonly use steam heated to 121 °C or 134 °C. To achieve sterility, a holding time of at least 15 minutes at 121 °C or 3 minutes at 134 °C is required. Additional sterilizing time is usually required for liquids and instruments packed in layers of cloth, as they may take longer to reach the required temperature. After sterilization, autoclaved liquids must be cooled slowly to avoid boiling over when the pressure is released.

Proper autoclave treatment will inactivate all fungi, bacteria, viruses and also bacterial spores, which can be quite resistant. It will not necessarily eliminate all prions.

For prion elimination, various recommendations state 121–132 °C (270 °F) for 60 minutes or 134 °C (273 °F) for at least 18 minutes. The prion that causes the disease scrapie (strain 263K) is inactivated relatively quickly by such sterilization procedures; however, other strains of scrapie, as well as strains of CJD and BSE are more resistant. Using mice as test animals, one experiment showed that heating BSE positive brain tissue at 134-138 °C (273-280 °F) for 18 minutes resulted in only a 2.5 log decrease in prion infectivity. (The initial BSE concentration in the tissue was relatively low). For a significant margin of safety, cleaning should reduce infectivity by 4 logs, and the sterilization method should reduce it a further 5 logs.

To ensure the autoclaving process was able to cause sterilization, most autoclaves have meters and charts that record or display pertinent information such as temperature and pressure as a function of time. Indicator tape is often placed on packages of products prior to autoclaving. A chemical in the tape will change color when the appropriate conditions have been met. Some types of packaging have built-in indicators on them.

Biological indicators ("bioindicators") can also be used to independently confirm autoclave performance. Simple bioindicator devices are commercially available based on microbial spores. Most contain spores of the heat resistant microbe *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*), among the toughest organisms for an autoclave to destroy. Typically these devices have a self-contained liquid growth medium and a growth indicator. After autoclaving an internal glass ampule is shattered, releasing the spores into the growth medium. The vial is then incubated (typically at 56 °C (132 °F)) for 24 hours. If the autoclave destroyed the spores, the medium will remain its original color. If autoclaving was unsuccessful the *B. sterothermophilus* will metabolize during incubation, causing a color change during the incubation.

For effective sterilization, steam needs to penetrate the autoclave load uniformly, so an autoclave must not be overcrowded, and the lids of bottles and containers must be left ajar. During the initial heating of the chamber, residual air must be removed. Indicators should be placed in the most difficult places for the steam to reach to ensure that steam actually penetrates there.

For autoclaving, as for all disinfection or sterilization methods, cleaning is critical. Extraneous biological matter or grime may shield organisms from the property intended to kill them, whether it physical or chemical. Cleaning can also remove a large number of organisms. Proper cleaning can be achieved by physical scrubbing. This should be done with detergent and warm water to get the best results. Cleaning instruments or utensils with organic matter, cool water must be used because warm or hot water may cause organic debris to coagulate. Treatment with ultrasound or pulsed air can also be used to remove debris.

## **Food**

Although imperfect, cooking and canning are the most common applications of heat sterilization. Boiling water kills the vegetative stage of all common microbes. Roasting meat until it is well done typically completely sterilizes the surface. Since the surface is also the part of food most likely to be contaminated by microbes, roasting usually prevents food poisoning. Note that the common methods of cooking food **do not** sterilize food - they simply reduce the number of disease-causing micro-organisms to a level that is not dangerous for people with normal digestive and immune systems.

Pressure cooking is analogous to autoclaving and when performed correctly renders food sterile. However, some foods are notoriously difficult to sterilize with home canning equipment, so expert recommendations should be followed for home processing to avoid food poisoning.

## **Food utensils**

Dishwashers often only use hot tap water or heat the water to between 49 and 60 °C (120 and 140 °F), and thus provide temperatures that could promote bacterial growth. That is to say, they do not effectively sterilize utensils. Some dishwashers do actually heat water up to 74 °C (165 °F) or higher; those often are specifically described as having sterilization modes of some sort, but this is not a substitute for autoclaving.

Note that dishwashers remove food traces from the utensils by a combination of mechanical action (the action of water hitting the plates and cutlery) and the action of detergents and

enzymes on fats and proteins. This removal of food particles thus removes one of the factors required for bacterial growth (food), it clearly explains why items with cracks and crevices should either be washed by hand or disposed of: if the water cannot get to the area needing cleaning, the warm, moist, dark conditions in the dishwasher can actually promote bacterial growth.

## **Bathing**

Bathing and washing are not hot enough to sterilize bacteria without scalding the skin. Most hot tap water is between 43 and 49 °C (110 and 120 °F), though some people set theirs as high as 55 °C (130 °F). Humans begin to find water painful at 41 to 42 °C (106 to 108 °F), which to many bacteria is just starting to get warm *enough* for them to grow quickly; they will grow faster, rather than be killed at temperatures up to 55 °C (130 °F) or more.

## **Other methods**

Other heat methods include flaming, incineration, boiling, tindalization, and using dry heat.

**Flaming** is done to loops and straight-wires in microbiology labs. Leaving the loop in the flame of a Bunsen burner or alcohol lamp until it glows red ensures that any infectious agent gets inactivated. This is commonly used for small metal or glass objects, but not for large objects (see Incineration below). However, during the initial heating infectious material may be "sprayed" from the wire surface before it is killed, contaminating nearby surfaces and objects. Therefore, special heaters have been developed that surround the inoculating loop with a heated cage, ensuring that such sprayed material does not further contaminate the area. Another problem is that gas flames may leave residues on the object, e.g. carbon, if the object is not heated enough.

A variation on flaming is to dip the object in 70% ethanol (or a higher concentration) and merely touch the object briefly to the Bunsen burner flame, but not hold it in the gas flame. The ethanol will ignite and burn off in a few seconds. 70% ethanol kills many, but not all, bacteria and viruses, and has the advantage that it leaves less residue than a gas flame. This method works well for the glass "hockey stick"-shaped bacteria spreaders.



**Incineration** will also burn any organism to ash. It is used to sanitize medical and other biohazardous waste before it is discarded with non-hazardous waste.

**Boiling in water** for fifteen minutes will kill most vegetative bacteria and inactivate viruses, but boiling is ineffective against prions and many bacterial and fungal spores; therefore boiling is unsuitable for sterilization. However, since boiling does kill most vegetative microbes and viruses, it is useful for reducing viable levels if no better method is available. Boiling is a simple process, and is an option available to most people, requiring only water, enough heat, and a container that can withstand the heat; however, boiling can be hazardous and cumbersome.

**Tindalization**<sup>[4]</sup> / **Tyndallization**<sup>[5]</sup> named after John Tyndall is a lengthy process designed to reduce the level of activity of sporulating bacteria that are left by a simple boiling water method. The process involves boiling for a period (typically 20 minutes) at atmospheric pressure, cooling, incubating for a day, boiling, cooling, incubating for a day, boiling, cooling, incubating for a day, and finally boiling again. The three incubation periods are to allow heat-resistant spores surviving the previous boiling period to germinate to form the heat-sensitive vegetative (growing) stage, which can be killed by the next boiling step. This is effective because many spores are stimulated to grow by the heat shock. The procedure only works for media that can support bacterial growth - it will not sterilize plain water. Tindalization/tyndallization is ineffective against prions.

**Dry heat** can be used to sterilize items, but as the heat takes much longer to be transferred to the organism, both the time and the temperature must usually be increased, unless forced ventilation of the hot air is used. The standard setting for a hot air oven is at least two hours at 160 °C (320 °F). A rapid method heats air to 190 °C (374 °F) for 6 minutes for unwrapped objects and 12 minutes for wrapped objects.<sup>[6][7]</sup> Dry heat has the advantage that it can be used on powders and other heat-stable items that are adversely affected by steam (for instance, it does not cause rusting of steel objects).

**Prions** can be inactivated by immersion in sodium hydroxide (NaOH 0.09N) for two hours plus one hour autoclaving (121 °C/250 °F). Several investigators have shown complete (>7.4

logs) inactivation with this combined treatment. However, sodium hydroxide may corrode surgical instruments, especially at the elevated temperatures of the autoclave.

**Glass bead sterilizer**, once a common sterilization method employed in dental offices as well as biologic laboratories,<sup>[8]</sup> is not approved by the U.S. Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) to be used as inter-patients sterilizer since 1997.<sup>[9]</sup> Still it is popular in European as well as Israeli dental practice although there are no current evidence-based guidelines for using this sterilizer.<sup>[8]</sup>

### **3. Chemical sterilization**

Chemicals are also used for sterilization. Although heating provides the most reliable way to rid objects of all transmissible agents, it is not always appropriate, because it will damage heat-sensitive materials such as biological materials, fiber optics, electronics, and many plastics. Low temperature gas sterilizers function by exposing the articles to be sterilized to high concentrations (typically 5 - 10% v/v) of very reactive gases (alkylating agents such as ethylene oxide, and oxidizing agents such as hydrogen peroxide and ozone). Liquid sterilants and high disinfectants typically include oxidizing agents such as hydrogen peroxide and peracetic acid and aldehydes such as glutaraldehyde and more recently o-phthalaldehyde. While the use of gas and liquid chemical sterilants/high level disinfectants avoids the problem of heat damage, users must ensure that article to be sterilized is chemically compatible with the sterilant being used. The manufacturer of the article can provide specific information regarding compatible sterilants. In addition, the use of chemical sterilants poses new challenges for workplace safety. The chemicals used as sterilants are designed to destroy a wide range of pathogens and typically the same properties that make them good sterilants makes them harmful to humans. Employers have a duty to ensure a safe work environment (Occupational Safety and Health Act of 1970, section 5 for United States) and work practices, engineering controls and monitoring should be employed appropriately.

#### **3.1. Ethylene Oxide**

**Ethylene oxide** (EO or EtO) gas is commonly used to sterilize objects sensitive to temperatures greater than 60 °C such as plastics, optics and electrics. Ethylene oxide

treatment is generally carried out between 30 °C and 60 °C with relative humidity above 30% and a gas concentration between 200 and 800 mg/L for at least three hours. Ethylene oxide penetrates well, moving through paper, cloth, and some plastic films and is highly effective. Ethylene oxide sterilizers are used to process sensitive instruments which cannot be adequately sterilized by other methods. EtO can kill all known viruses, bacteria and fungi, including bacterial spores and is satisfactory for most medical materials, even with repeated use. However it is highly flammable, and requires a longer time to sterilize than any heat treatment. The process also requires a period of post-sterilization aeration to remove toxic residues. Ethylene oxide is the most common sterilization method, used for over 70% of total sterilizations, and for 50% of all disposable medical devices.

The two most important ethylene oxide sterilization methods are: (1) the gas chamber method and (2) the micro-dose method. To benefit from economies of scale, EtO has traditionally been delivered by flooding a large chamber with a combination of EtO and other gases used as dilutants (usually CFCs or carbon dioxide ). This method has drawbacks inherent to the use of large amounts of sterilant being released into a large space, including air contamination produced by CFCs and/or large amounts of EtO residuals, flammability and storage issues calling for special handling and storage, operator exposure risk and training costs

Because of these problems a micro-dose sterilization method was developed in the late 1950s, using a specially designed bag to eliminate the need to flood a larger chamber with EtO. This method is also known as gas diffusion sterilization, or bag sterilization. This method minimizes the use of gas.<sup>[10]</sup>

### **3.2. Spore testing**

*Bacillus atrophaeus*, (reclassified from *Bacillus subtilis*), a very resistant organism, is used as a rapid biological indicator for EO sterilizers. If sterilization fails, incubation at 37 °C causes a fluorescent change within four hours, which is read by an auto-reader. After 96 hours, a visible color change occurs. Fluorescence is emitted if a particular (EO resistant) enzyme is present, which means that spores are still active. The color change indicates a pH shift due to

bacterial metabolism. The rapid results mean that the objects treated can be quarantined until the test results are available.

### **3.3. Ozone**

Ozone is used in industrial settings to sterilize water and air, as well as a disinfectant for surfaces. It has the benefit of being able to oxidize most organic matter. On the other hand, it is a toxic and unstable gas that must be produced on-site, so it is not practical to use in many settings.

Ozone offers many advantages as a sterilant gas; ozone is a very efficient sterilant because of its strong oxidizing properties ( $E = 2.076$  vs SHE, CRC Handbook of Chemistry and Physics, 76th Ed, 1995-1996) capable of destroying a wide range of pathogens, including prions[2] without the need for handling hazardous chemicals since the ozone is generated within the sterilizer from medical grade oxygen. In 2005 a Canadian company called TSO3 Inc[3] received FDA clearance to sell an ozone sterilizer for use in healthcare. The high reactivity of ozone means that waste ozone can be destroyed by passing over a simple catalyst that reverts it back to oxygen and also means that the cycle time is relatively short (about 4.5 hours for TSO3's model 125L). The downside of using ozone is that the gas is very reactive and very hazardous. The NIOSH immediately dangerous to life and health limit for ozone is 5 ppm, much 160 times smaller than the 800 ppm IDLH for ethylene oxide.Documentation for Immediately Dangerous to Life or Health Concentrations (IDLH): NIOSH Chemical Listing and Documentation of Revised IDLH Values (as of 3/1/95) and OSHA has set the PEL for ozone at 0.1 ppm calculated as an eight hour time weighted average (29 CFR 1910.1000, Table Z-1). The Canadian Center for Occupation Health and Safety provides an excellent summary of the health effects of exposure to ozone.[4] The sterilant gas manufacturers include many safety features in their products but prudent practice is to provide continuous monitoring to below the OSHA PEL to provide a rapid warning in the even of a leak and monitors for determining workplace exposure to ozone are commercially available.

### **3.4. Bleach**

**Chlorine bleach** is another accepted liquid sterilizing agent. Household bleach consists of 5.25% sodium hypochlorite. It is usually diluted to 1/10 immediately before use; however to kill Mycobacterium tuberculosis it should be diluted only 1/5, and 1/2.5 (1 part bleach and 1.5 parts water) to inactivate prions. The dilution factor must take into account the volume of any liquid waste that it is being used to sterilize.<sup>[11]</sup> Bleach will kill many organisms immediately, but for full sterilization it should be allowed to react for 20 minutes. Bleach will kill many, but not all spores. It is highly corrosive and may corrode even stainless steel surgical instruments.

Bleach decomposes over time when exposed to air, so fresh solutions should be made daily.  
[12]

### 3.5. Glutaraldehyde and Formaldehyde

**Glutaraldehyde and formaldehyde** solutions (also used as fixatives) are accepted liquid sterilizing agents, provided that the immersion time is sufficiently long. To kill all spores in a clear liquid can take up to 12 hours with glutaraldehyde and even longer with formaldehyde. The presence of solid particles may lengthen the required period or render the treatment ineffective. Sterilization of blocks of tissue can take much longer, due to the time required for the fixative to penetrate. Glutaraldehyde and formaldehyde are volatile, and toxic by both skin contact and inhalation. Glutaraldehyde has a short shelf life (<2 weeks), and is expensive. Formaldehyde is less expensive and has a much longer shelf life if some methanol is added to inhibit polymerization to paraformaldehyde, but is much more volatile. Formaldehyde is also used as a gaseous sterilizing agent; in this case, it is prepared on-site by depolymerization of solid paraformaldehyde. Many vaccines, such as the original Salk polio vaccine, are sterilized with formaldehyde.

### 3.6. Phthalaldehyde

**Ortho-phthalaldehyde** (OPA) is a chemical sterilizing agent that received Food and Drug Administration (FDA) clearance in late 1999. Typically used in a 0.55% solution, OPA shows better myco-bactericidal activity than glutaraldehyde. It also is effective against glutaraldehyde-resistant spores. OPA has superior stability, is less volatile, and does not

irritate skin or eyes, and it acts more quickly than glutaraldehyde. On the other hand, it is more expensive, and will stain proteins (including skin) gray in color.

### **3.7. Hydrogen Peroxide**

**Hydrogen peroxide** is another chemical sterilizing agent. It is relatively non-toxic when diluted to low concentrations, such as the familiar 3 % retail solutions although hydrogen peroxide is a dangerous oxidizer at high concentrations (> 10% w/w). Hydrogen peroxide is strong oxidant and these oxidizing properties allow it to destroy a wide range of pathogens and it is used to sterilize heat or temperature sensitive articles such as rigid endoscopes. In medical sterilization hydrogen peroxide is used at higher concentrations, ranging from around 35 % up to 90%. The biggest advantage of hydrogen peroxide as a sterilant is the short cycle time. Whereas the cycle time for ethylene oxide (discussed above) may be 10 to 15 hours, the use of very high concentrations of hydrogen peroxide allows much shorter cycle times. Some hydrogen peroxide modern sterilizers, such as the Sterrad NX have a cycle time as short as 28 minutes.

Hydrogen peroxide sterilizers have their drawbacks. Since hydrogen peroxide is a strong oxidant, there are material compatibility issues and users should consult the manufacturer of the article to be sterilized to ensure that it is compatible with this method of sterilization. Paper products cannot be sterilized in the Sterrad system because of a process called cellulostics, in which the hydrogen peroxide would be completely absorbed by the paper product. The penetrating ability of hydrogen peroxide is not as good as ethylene oxide and so there are limitations on the length and diameter of lumens that can be effectively sterilized and guidance is available from the sterilizer manufacturers.

While hydrogen peroxide offers significant advantages in terms of throughput, as with all sterilant gases, sterility is achieved through the use of high concentrations of reactive gases. Hydrogen peroxide is primary irritant and the contact of the liquid solution with skin will cause bleaching or ulceration depending on the concentration and contact time. The vapor is also hazardous with the target organs being the eyes and respiratory system. Even short term exposures can be hazardous and NIOSH has set the Immediately Dangerous to Life and

Health Level (IDLH) at 75 ppm.[5], less than one tenth the IDLH for ethylene oxide (800 ppm). Prolonged exposure to even low ppm concentrations can cause permanent lung damage and consequently OSHA has set the permissible exposure limit to 1.0 ppm, calculated as an 8 hour time weighted average (29 CFR 1910.1000 Table Z-1). Employers thus have a legal duty to ensure that their personnel are not exposed to concentrations exceeding this PEL. Even though the sterilizer manufacturers go to great lengths to make their products safe through careful design and incorporation of many safety features, workplace exposures of hydrogen peroxide from gas sterilizers are documented in the FDA MAUDE database[6]. When using any type of gas sterilizer, prudent work practices will include good ventilation (10 air exchanges per hour), a continuous gas monitor for hydrogen peroxide as well as good work practices and training. Further information about the health effects of hydrogen peroxide and good work practices is available from OSHA[7] and the ATSDR.[8]

Hydrogen peroxide can also be mixed with formic acid as needed in the Endoclen device for sterilization of endoscopes. This device has two independent asynchronous bays, and cleans (in warm detergent with pulsed air), sterilizes and dries endoscopes automatically in 30 minutes. Studies with synthetic soil with bacterial spores showed the effectiveness of this device.

#### **4. Dry sterilization process**

**Dry sterilization process** (DSP) uses hydrogen peroxide at a concentration of 30-35% under low pressure conditions. This process achieves bacterial reduction of  $10^{-6}$ ... $10^{-8}$ . The complete process cycle time is just 6 seconds, and the surface temperature is increased only 10-15 °C (18 to 27 °F). Originally designed for the sterilization of plastic bottles in the beverage industry, because of the high germ reduction and the slight temperature increase the dry sterilization process is also useful for medical and pharmaceutical applications.

#### **Peracetic acid**

**Peracetic acid** (0.2%) is used to sterilize instruments in the Steris system.

## Prions

**Prions** are highly resistant to chemical sterilization. Treatment with aldehydes (e.g., formaldehyde) have actually been shown to increase prion resistance. Hydrogen peroxide (3%) for one hour was shown to be ineffective, providing less than 3 logs ( $10^{-3}$ ) reduction in contamination. Iodine, formaldehyde, glutaraldehyde and peracetic acid also fail this test (one hour treatment). Only chlorine, a phenolic compound, guanidinium thiocyanate, and sodium hydroxide (NaOH) reduce prion levels by more than 4 logs. Chlorine and NaOH are the most consistent agents for prions. Chlorine is too corrosive to use on certain objects. Sodium hydroxide has had many studies showing its effectiveness.

## Silver

Silver ions and silver compounds show a toxic effect on some bacteria, viruses, algae and fungi, typical for heavy metals like lead or mercury, but without the high toxicity to humans that is normally associated with these other metals. Its germicidal effects kill many microbial organisms *in vitro*, but testing and standardization of silver products is yet difficult.<sup>[13]</sup>

Hippocrates, the father of modern medicine, wrote that silver had beneficial healing and anti-disease properties<sup>[cite this quote]</sup>, and the Phoenicians used to store water, wine, and vinegar in silver bottles to prevent spoiling. In the early 1900s people would put silver dollars in milk bottles to prolong the milk's freshness.<sup>[14]</sup> The exact process of silver's germicidal effect is still not well understood. One of the explanations is the oligodynamic effect, which accounts for the effect on microorganisms but not on virii.

Silver compounds were used to prevent infection in World War I before the advent of antibiotics. Silver nitrate solution was a standard of care but was largely replaced by silver sulfadiazine cream (SSD Cream),<sup>[15]</sup> which was generally the "standard of care" for the antibacterial and antibiotic treatment of serious burns until the late 1990s.<sup>[16]</sup> Now, other options, such as silver-coated dressings (activated silver dressings), are used in addition to SSD cream. However, the evidence for the use of such silver-treated dressings is mixed and although the evidence on if they are effective is promising, it is marred by the poor quality of the trials used to assess these products.<sup>[17]</sup> Consequently a major systematic review by the



Cochrane Collaboration found insufficient evidence to recommend the use of silver-treated dressings to treat infected wounds.<sup>[18]</sup>

The widespread use of silver went out of fashion with the development of antibiotics. However, recently there has been renewed interest in silver as a broad-spectrum antimicrobial. In particular, silver is being used with alginate, a naturally occurring biopolymer derived from seaweed, in a range of products designed to prevent infections as part of wound management procedures, particularly applicable to burn victims.<sup>[19]</sup> In 2007, AGC Flat Glass Europe introduced the first antibacterial glass to fight hospital-caught infection: it is covered with a thin layer of silver.<sup>[20]</sup> In addition, Samsung has introduced washing machines with a final rinse containing silver ions to provide several days of antibacterial protection in the clothes.<sup>[21]</sup> Kohler has introduced a line of toilet seats that have silver ions embedded to kill germs. A company called Thomson Research Associates has begun treating products with Ultra Fresh, an anti-microbial technology involving "proprietary nano-technology to produce the ultra-fine silver particles essential to ease of application and long-term protection."<sup>[22]</sup> The U.S. Food and Drug Administration (FDA) has recently approved an endotracheal breathing tube with a fine coat of silver for use in mechanical ventilation, after studies found it reduced the risk of ventilator-associated pneumonia.<sup>[23]</sup>

It has long been known that antibacterial action of silver is enhanced by the presence of an electric field. Applying a few volts of electricity across silver electrodes drastically enhances the rate that bacteria in solution are killed. It was found recently that the antibacterial action of silver electrodes is greatly improved if the electrodes are covered with silver nanorods.<sup>[24]</sup> Note that enhanced antibacterial properties of nanoparticles compared to bulk material is not limited to silver, but has also been demonstrated on other materials such as ZnO.<sup>[25]</sup>

## **5. Radiation Sterilization**

Methods of sterilization exist using radiation such as electron beams, X-rays, gamma rays, or subatomic particles.<sup>[26]</sup>

- Gamma rays are very penetrating and are commonly used for sterilization of disposable medical equipment, such as syringes, needles, cannulas and IV sets.

Gamma radiation requires bulky shielding for the safety of the operators; they also require storage of a radioisotope (usually Cobalt-60), which continuously emits gamma rays (it cannot be turned off, and therefore always presents a hazard in the area of the facility).

- Electron beam processing is also commonly used for medical device sterilization. Electron beams use an on-off technology and provide a much higher dosing rate than gamma or x-rays. Due to the higher dose rate, less exposure time is needed and thereby any potential degradation to polymers is reduced. A limitation is that electron beams are less penetrating than either gamma or x-rays.
- X-rays, if low energy, are less penetrating than gamma rays and tend to require longer exposure times, but require less shielding. They are generated by an X-ray machine that can be turned off for servicing and when not in use.
- Ultraviolet light irradiation (UV, from a germicidal lamp) is useful only for sterilization of surfaces and some transparent objects. Many objects that are transparent to visible light absorb UV. UV irradiation is routinely used to sterilize the interiors of biological safety cabinets between uses, but is ineffective in shaded areas, including areas under dirt (which may become polymerized after prolonged irradiation, so that it is very difficult to remove). It also damages many plastics, such as polystyrene foam.

*Further information: Ultraviolet Germicidal Irradiation*

- Subatomic particles may be more or less penetrating, and may be generated by a radioisotope or a device, depending upon the type of particle.

Irradiation with X-rays or gamma rays does not make materials radioactive. Irradiation with particles may make materials radioactive, depending upon the type of particles and their energy, and the type of target material: neutrons and very high-energy particles can make materials radioactive, but have good penetration, whereas lower energy particles (other than neutrons) cannot make materials radioactive, but have poorer penetration.

Irradiation is used by the United States Postal Service to sterilize mail in the Washington, DC area. Some foods (e.g. spices, ground meats) are irradiated for sterilization (see food irradiation).

## 6. Sterile filtration

Clear liquids that would be damaged by heat, irradiation or chemical sterilization can be sterilized by mechanical filtration. This method is commonly used for sensitive pharmaceuticals and protein solutions in biological research. A filter with pore size 0.2  $\mu\text{m}$  will effectively remove bacteria. If viruses must also be removed, a much smaller pore size around 20  $\text{nm}$  is needed. Solutions filter slowly through membranes with smaller pore diameters. Prions are not removed by filtration. The filtration equipment and the filters themselves may be purchased as presterilized disposable units in sealed packaging, or must be sterilized by the user, generally by autoclaving at a temperature that does not damage the fragile filter membranes. To ensure sterility, the filtration system must be tested to ensure that the membranes have not been punctured prior to or during use.

To ensure the best results, pharmaceutical sterile filtration is performed in a room with highly filtered air (HEPA filtration) or in a laminar flow cabinet or "flowbox", a device which produces a laminar stream of HEPA filtered air.

## 7. Antibacterial soap

**Antibacterial soap** is any cleaning product to which active antibacterial ingredients have been added. These chemicals kill bacteria and microbes. They do not kill viruses.

### 7.1. Ingredients

All liquid hand and body soaps contain antibacterial chemicals. Triclosan is a common ingredient, as is alcohol. Since there is a great variety of bacteria, effectiveness against any given type of bacterium does not ensure that it is effective against unrelated types. These are generally only contained at preservative level unless the product is marked antibacterial, antiseptic, or germicidal. Triclosan, Triclocarban/Trichlorocarbamide and

PCMX/Chloroxylenol are commonly used for antibacterial and deodorant effect in consumer products.

Some soaps contain tetrasodium EDTA which is a chelating agent that sequesters metals that the bacteria require in order to grow. Other microbes also require metals and so it is actually an anti-microbial agent that is widely used even as a preservative. It appears to be fairly harmless in the environment.

## **Overuse**

Overuse of chemicals like triclosan has been suggested to cause sensitive bacteria to evolve resistance to its antibacterial action. Should any antibiotic be discovered that works similarly to triclosan, this antibiotic's effectiveness to combat infections will be reduced because people will be hosting resistant bacteria already due to their use of soaps containing triclosan.

## **Research**

Studies have examined the purported benefits of antibacterial soap.<sup>[citation needed]</sup> Some studies have concluded that simply washing thoroughly with plain soap is sufficient to reduce bacteria and, further, is effective against viruses. Other studies have found that soaps containing antimicrobial active ingredients remove more bacteria than simply washing with plain soap and water (*J.C. Lucet (2002), Hand Contamination Before and After Different Hand Hygiene Techniques: a Randomized Clinical Trial, Journal of Hospital Infection; L.L. Gibson (2002), Quantitative Assessment of Risk Reduction From Hand Washing with Antibacterial Soap, Journal of Applied Microbiology*). The U.S. Food and Drug Administration published reports that questions the use of antibacterial soap and hand sanitizers saying that it found no medical studies that showed a link between a specific consumer antibacterial product and a decline in infection rates.<sup>[1]</sup>

At one conference, Dr. Stuart Levy, a microbiologist at Tufts University, cites these studies to conclude: "Dousing everything we touch with antibacterial soaps and taking antibiotic medications at the first sign of a cold can upset the natural balance of microorganisms in and around us, leaving behind only the 'superbugs'."<sup>1</sup>

In addition, the use of antibacterial soaps when suffering from superficial mycoses can greatly enhance the pathogenicity of the fungus in question by removing potential bacterial competition, causing a rapid increase in fungal growth.<sup>[citation needed]</sup>

Recent research from Dr. Levy's lab (Aiello, et al., 2005) concludes that "The results from our study do not implicate the use of antibacterial cleaning and hygiene products as an influential factor in carriage of antimicrobial drug-resistant bacteria on the hands of household members." However, a more recent literature review performed by Dr. Levy (Aiello et al., 2007) concluded that "The lack of an additional health benefit associated with the use of triclosan-containing consumer soaps over regular soap, coupled with laboratory data demonstrating a potential risk of selecting for drug resistance, warrants further evaluation by governmental regulators regarding antibacterial product claims and advertising." The paper's authors called for continued research in this area.

## 7. Antiseptic



An antiseptic solution of Povidone-iodine applied to an abrasion

**Antiseptics** (from Greek *avtí* - *anti*, "against" + *σηπτικός* - *septikos*, "putrefactive") are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction. They should generally be distinguished from antibiotics that destroy bacteria within the body, and from disinfectants, which destroy microorganisms found on non-living objects. Some antiseptics are true germicides, capable of destroying microbes (bacteriocidal), whilst others are bacteriostatic and only prevent or inhibit their

growth. **Antibacterials** are antiseptics that only act against bacteria. Microbicides which kill virus particles are called viricides.

### 7.1. Usage in surgery

The widespread introduction of antiseptic surgical methods followed the publishing of the paper *Antiseptic Principle of the Practice of Surgery* in 1867 by Joseph Lister, inspired by Louis Pasteur's germ theory of putrefaction. In this paper he advocated the use of carbolic acid (phenol) as a method of ensuring that any germs present were killed. Some of this work was anticipated by:

- Dr. George H Tichenor who experimented with the use of alcohol on wounds ca. 1861-1863, and subsequently marketed a product for this purpose known as "Dr. Tichenor's Patent Medicine " after the American Civil War.
- Ignaz Semmelweis who published his work "The Cause, Concept and Prophylaxis of Childbed Fever" in 1861, summarizing experiments and observations since 1847.<sup>[1]</sup>
- Florence Nightingale, who contributed substantially to the report on the Royal Commission on the Health of the Army (1856–1857), based on her earlier work
- Oliver Wendell Holmes, Sr., who published "The Contagiousness of Puerperal Fever" in 1843.

and even the ancient Greek physicians Galen (ca 130–200 AD) and Hippocrates (ca 400 BC). There is even a Sumerian clay tablet dating from 2150 BC advocating the use of similar techniques.<sup>[2]</sup>

But every antiseptic, however good, is more or less toxic and irritating to a wounded surface. Hence it is that the antiseptic method has been replaced in the surgery of today by the aseptic method, which relies on keeping free from the invasion of bacteria rather than destroying them when present.

### 7.2. How it works

For the growth of bacteria there must be a food supply, moisture, in most cases oxygen, and a certain minimum temperature (see bacteriology). These conditions have been studied and applied in preserving of food and the ancient practice of embalming the dead, which is the earliest known systematic use of antiseptics.

In early inquiries, there was much emphasis on the prevention of putrefaction, and procedures were carried out to find how much of an agent must be added to a given solution in order to prevent development of undesirable bacteria. However, for various reasons, this method was inaccurate, and today an antiseptic is judged by its effect on pure cultures of defined pathogenic cellular single helix microbes and their vegetative and spore forms. The standardization of antiseptics has been implemented in many instances, and a water solution of phenol of a certain fixed strength is now used as the standard to which other antiseptics are compared.

### 7.3. Some common antiseptics

- Alcohols

Most commonly used are ethanol (60-90%), 1-propanol (60-70%) and 2-propanol/isopropanol (70-80%) or mixtures of these alcohols. They are commonly referred to as "surgical alcohol". Used to disinfect the skin before injections are given, often along with iodine (tincture of iodine) or some cationic surfactants (benzalkonium chloride 0.05 - 0.5%, chlorhexidine 0.2 - 4.0% or octenidine dihydrochloride 0.1 - 2.0%).

- Quaternary ammonium compounds

Also known as *Quats* or *QAC's*, include the chemicals benzalkonium chloride (BAC), cetyl trimethylammonium bromide (CTMB), cetylpyridinium chloride (Cetrim, CPC) and benzethonium chloride (BZT). Benzalkonium chloride is used in some pre-operative skin disinfectants (conc. 0.05 - 0.5%) and antiseptic towels. The antimicrobial activity of Quats is inactivated by anionic surfactants, such as soaps. Related disinfectants include chlorhexidine and octenidine.

- Boric acid

Used in suppositories to treat yeast infections of the vagina, in eyewashes, and as an antiviral to shorten the duration of cold sore attacks. Put into creams for burns. Also common in trace amounts in eye contact solution. Though it is popularly known as an antiseptic, it is in reality only a soothing fluid, and bacteria will flourish comfortably in contact with it. <sup>[citation needed]</sup>

- Chlorhexidine Gluconate

A biguanidine derivative, used in concentrations of 0.5 - 4.0% alone or in lower concentrations in combination with other compounds, such as alcohols. Used as a skin antiseptic and to treat inflammation of the gums (gingivitis). The microbicidal action is somewhat slow, but remanent. It is a cationic surfactant, similar to Quats.

- Hydrogen peroxide

Used as a 6% (20Vols) solution to clean and deodorize wounds and ulcers. More common 1% or 2% solutions of hydrogen peroxide have been used in household first aid for scrapes, etc. However, even this less potent form is no longer recommended for typical wound care as the strong oxidization causes scar formation and increases healing time. Gentle washing with mild soap and water or rinsing a scrape with sterile saline is a better practice.

- Iodine

Usually used in an alcoholic solution (called tincture of iodine) or as Lugol's iodine solution as a pre- and post-operative antiseptic. No longer recommended to disinfect minor wounds because it induces scar tissue formation and increases healing time. Gentle washing with mild soap and water or rinsing a scrape with sterile saline is a better practice. Novel iodine antiseptics containing povidone-iodine (an iodophor, complex of povidone, a water-soluble polymer, with triiodide anions  $I_3^-$ , containing about 10% of active iodine) are far better tolerated, don't affect wound healing



negatively and leave a deposit of active iodine, creating the so-called "remanent," or persistent, effect. The great advantage of iodine antiseptics is the widest scope of antimicrobial activity, killing all principal pathogens and given enough time even spores, which are considered to be the most difficult form of microorganisms to be inactivated by disinfectants and antiseptics.

- Mercurochrome

Not recognized as safe and effective by the U.S. Food and Drug Administration (FDA) due to concerns about its mercury content. Other obsolete organomercury antiseptics include bis-(phenylmercuric) monohydrogenborate (Famosept).

- Octenidine dihydrochloride

A cationic surfactant and bis-(dihydropyridinyl)-decane derivative, used in concentrations of 0.1 - 2.0%. It is similar in its action to the Quats, but is of somewhat broader spectrum of activity. Octenidine is currently increasingly used in continental Europe as a QAC's and chlorhexidine (with respect to its slow action and concerns about the carcinogenic impurity 4-chloroaniline) substitute in water- or alcohol-based skin, mucosa and wound antiseptic. In aqueous formulations, it is often potentiated with addition of 2-phenoxyethanol.

- Phenol (carbolic acid) compounds

Phenol is germicidal in strong solution, inhibitory in weaker ones. Used as a "scrub" for pre-operative hand cleansing. Used in the form of a powder as an antiseptic baby powder, where it is dusted onto the navel as it heals. Also used in mouthwashes and throat lozenges, where it has a painkilling effect as well as an antiseptic one. Example: TCP. Other phenolic antiseptics include historically important, but today rarely used (sometimes in dental surgery) thymol, today obsolete hexachlorophene, still used triclosan and sodium 3,5-dibromo-4-hydroxybenzenesulfonate (Dibromol).

- Sodium chloride

Used as a general cleanser. Also used as an antiseptic mouthwash. Only a weak antiseptic effect, due to hyperosmolality of the solution above 0.9%.

- Sodium hypochlorite

Used in the past, diluted, neutralized and combined with potassium permanganate in the Daquin's solution. It is now used only as disinfectant.

- Calcium hypochlorite

Used by Semmelweis, as "chlorinated lime", in his revolutionary efforts against childbed fever.

- Sodium bicarbonate (NaHCO<sub>3</sub>)

has antiseptic and disinfectant properties. <sup>[3][4]</sup>

### **Negative effects**

Stuart B. Levy, in a presentation to the 2000 Emerging Infectious Diseases Conference, expressed concern that the overuse of antiseptic and antibacterial agents might lead to an increase in dangerous, resistant strains of bacteria.<sup>[5]</sup> The theory states that this could cause bacteria to evolve to the point where they are no longer harmed by antiseptics.

### **Endogenous**

The body produces its own antiseptics, which are a part of the chemical barriers of the immune system. The skin and respiratory tract secrete antimicrobial peptides such as the  $\beta$ -defensins.<sup>[6]</sup> Enzymes such as lysozyme and phospholipase A2 in saliva, tears, and breast milk are also antiseptic.<sup>[7][8]</sup> Vaginal secretions serve as a chemical barrier following menarche, when they become slightly acidic, while semen contains defensins and zinc to kill pathogens.<sup>[9][10]</sup> In the stomach, gastric acid and proteases serve as powerful chemical defenses against ingested pathogens.

## **8. Antibiotic resistance**

**Antibiotic resistance** is the ability of a microorganism to withstand the effects of antibiotics. It is a specific type of drug resistance. Antibiotic resistance evolves via natural selection acting upon random mutation, but it can also be engineered by applying an evolutionary stress on a population. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals) by plasmid exchange. If a bacterium carries several resistance genes, it is called **multiresistant** or, informally, a **superbug**. The term **antimicrobial resistance** is sometimes used to explicitly encompass organisms other than bacteria.

Antibiotic resistance can also be introduced artificially into a microorganism through transformation protocols. This can aid in implanting artificial genes into the microorganism. If the resistance gene is linked with the gene to be implanted, the antibiotic can be used to kill off organisms that lack the new gene.

#### D. MEDIUM KULTUR



An Agar Plate -- an example of a bacterial growth medium. Specifically, it is a streak plate; the orange lines and dots are formed by bacterial colonies.

A **growth medium** or **culture medium** is a liquid or gel designed to support the growth of microorganisms or cells <sup>[1]</sup>, or small plants like the moss *Physcomitrella patens* <sup>[2]</sup>. There are different types of media for growing different types of cells. <sup>[3]</sup>

There are two major types of growth media: those used for cell culture, which use specific cell types derived from plants or animals, and microbiological culture, which are used for growing microorganisms, such as bacteria or yeast. The most common growth media for microorganisms are *nutrient broths* and agar plates; specialized media are sometimes required for microorganism and cell culture growth. <sup>[1]</sup> Some organisms, termed *fastidious organisms*, require specialized environments due to complex nutritional requirements.

Viruses, for example, are obligatory intracellular parasites and require a growth medium composed of living cells.

An **agar plate** is a sterile Petri dish that contains a growth medium (typically agar plus nutrients) used to culture microorganisms or small plants like the moss *Physcomitrella patens*. Selective growth compounds may also be added to the media, such as antibiotics.<sup>[1]</sup>

Individual microorganisms placed on the plate will grow into individual colonies, each a clone genetically identical to the individual ancestor organism (except for the low, unavoidable rate of mutation). Thus, the plate can be used either to estimate the concentration of organisms in a liquid culture or a suitable dilution of that culture using a colony counter, or to generate genetically pure cultures from a mixed culture of genetically different organisms, using a technique known as "streaking". In this technique, a drop of the culture on the end of a thin, sterile loop of wire is streaked across the surface of the agar leaving organisms behind, a higher number at the beginning of the streak and a lower number at the end. At some point during a successful "streak", the number of organisms deposited will be such that distinct individual colonies will grow in that area which may be removed for further culturing, using another sterile loop.<sup>[1]</sup>

### **Types of growth media**

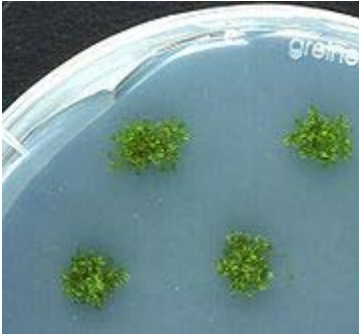
The most common growth media for microorganisms are *nutrient broths* (liquid nutrient medium) or *Luria Bertani medium* (LB medium or *Lysogeny Broth*). Liquid media are often mixed with agar and poured into petri dishes to solidify. These agar plates provide a solid medium on which microbes may be cultured. Bacteria grown in liquid cultures often form colloidal suspensions.

The differences between growth media used for cell culture and those used for microbiological culture are because cells derived from whole organisms and grown in culture often cannot grow without the addition of, for instance, hormones or growth factors which usually occur *in vivo*.<sup>[4]</sup> In the case of animal cells, this difficulty is often addressed by the addition of blood serum to the medium. In the case of microorganisms, there are no such limitations, as they are often unicellular organisms. One other major difference is that animal cells in culture are often grown on a flat surface to which they attach, and the medium is provided in a liquid form, which covers the cells. In contrast, bacteria such as *Escherichia coli* may be grown on solid media or in liquid media.

An important distinction between growth media types is that of *defined* versus *undefined* media.<sup>[1]</sup> A defined medium will have known quantities of all ingredients. For microorganisms, they consist of providing trace elements and vitamins required by the microbe and especially a defined carbon source and nitrogen source. Glucose or glycerol are often used as carbon sources, and ammonium salts or nitrates as inorganic nitrogen sources). An undefined medium has some complex ingredients, such as yeast extract or casein hydrolysate, which consist of a mixture of many, many chemical species in unknown proportions. Undefined media are sometimes chosen based on price and sometimes by necessity - some microorganisms have never been cultured on defined media.

A good example of a growth medium is the wort used to make beer. The wort contains all the nutrients required for yeast growth, and under anaerobic conditions, alcohol is produced. When the fermentation process is complete, the combination of medium and dormant microbes, now beer, is ready for consumption.

### **Nutrient media**



*Physcomitrella patens* plants growing axenically on agar plates (Petri dish, 9 cm diameter).

An *undefined medium* (also known as a *basal* or *complex* medium) is a medium that contains:

- a carbon source such as glucose for bacterial growth
- water
- various salts needed for bacterial growth
- a source of amino acids and nitrogen (e.g., beef, yeast extract)

This is an undefined medium because the amino acid source contains a variety of compounds with the exact composition being unknown. Nutrient media contain all the elements that most bacteria need for growth and are non-selective, so they are used for the general cultivation and maintenance of bacteria kept in laboratory culture collections.

*Defined media* (also known as chemically defined media)

- all the chemicals used are known
- does not contain any animal, yeast, plant tissue.

*Differential medium*

- some sort of indicator, typically a dye, is added, that allows for the differentiation of particular chemical reactions occurring during growth.

### **Minimal media**

*Minimal media* are those that contain the minimum nutrients possible for colony growth, generally without the presence of amino acids, and are often used by microbiologists and

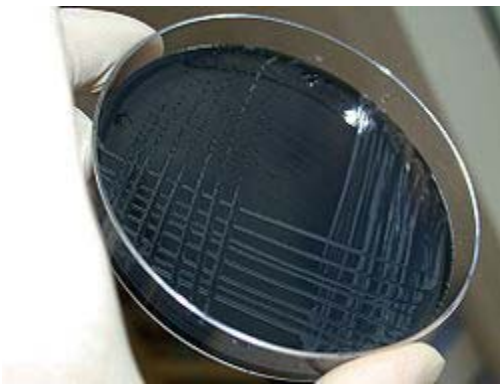
geneticists to grow "wild type" microorganisms. Minimal media can also be used to select for or against recombinants or exconjugants.

Minimal medium typically contains:

- a carbon source for bacterial growth, which may be a sugar such as glucose, or a less energy-rich source like succinate
- various salts, which may vary among bacteria species and growing conditions; these generally provide essential elements such as magnesium, nitrogen, phosphorus, and sulfur to allow the bacteria to synthesize protein and nucleic acid
- water

*Supplementary minimal media* are a type of minimal media that also contains a single selected agent, usually an amino acid or a sugar. This supplementation allows for the culturing of specific lines of auxotrophic recombinants.

### Selective media



Blood-free, charcoal-based selective medium agar (CSM) for isolation of Campylobacter.



Blood agar plates are often used to diagnose infection. On the right is a positive Streptococcus culture; on the left a positive Staphylococcus culture.

*Selective media* are used for the growth of only select microorganisms. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or tetracycline, then that antibiotic can be added to the medium in order to prevent other cells, which do not possess the resistance, from growing. Media lacking an amino acid such as proline in conjunction

with *E. coli* unable to synthesize it were commonly used by geneticists before the emergence of genomics to map bacterial chromosomes.

Selective growth media are also used in cell culture to ensure the survival or proliferation of cells with certain properties, such as antibiotic resistance or the ability to synthesize a certain metabolite. Normally, the presence of a specific gene or an allele of a gene confers upon the cell the ability to grow in the selective medium. In such cases, the gene is termed a marker.

Selective growth media for eukaryotic cells commonly contain neomycin to select cells that have been successfully transfected with a plasmid carrying the neomycin resistance gene as a marker. Gancyclovir is an exception to the rule as it is used to specifically kill cells that carry its respective marker, the Herpes simplex virus thymidine kinase (HSV TK).



Four types of agar plates demonstrating differential growth depending on bacterial metabolism.

Some examples of selective media include:

- eosin-methylen blue agar (EMB) that contains methylene blue – toxic to Gram-positive bacteria, allowing only the growth of Gram-negative bacteria
- YM (yeast and mold) which has a low pH, deterring bacterial growth
- blood agar (used in strep tests), which contains beef heart blood that becomes transparent in the presence of hemolytic Streptococcus
- MacConkey agar for Gram-negative bacteria
- Hektoen Enteric (HE) which is selective for Gram-negative bacteria
- Mannitol Salt Agar (MSA) which is selective for Gram-positive bacteria and differential for mannitol
- Terrific Broth (TB) is used with glycerol in cultivating recombinant strains of *Escherichia coli*.
- xylose lysine desoxyscholate (XLD), which is selective for Gram-negative bacteria

- Buffered charcoal yeast extract agar, which is selective for certain gram-negative bacteria, especially *Legionella pneumophila*

### **Differential media**

*Differential media* or *indicator media* distinguish one microorganism type from another growing on the same media.<sup>[5]</sup> This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red, eosin y, or methylene blue) added to the medium to visibly indicate the defining characteristics of a microorganism. This type of media is used for the detection of microorganisms and by molecular biologists to detect recombinant strains of bacteria.

Examples of differential media include:

- Eosin methylene blue (EMB), which is differential for lactose and sucrose fermentation
- MacConkey (MCK), which is differential for lactose fermentation
- Mannitol Salt Agar (MSA), which is differential for mannitol fermentation
- X-gal plates, which are differential for lac operon mutants