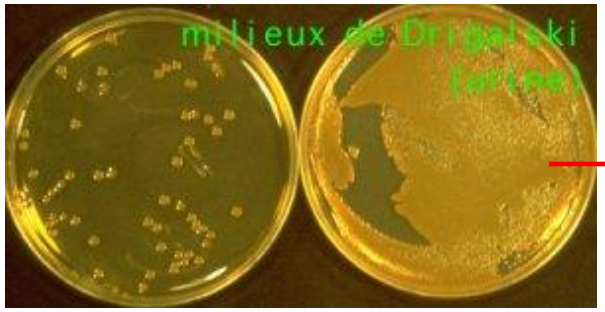
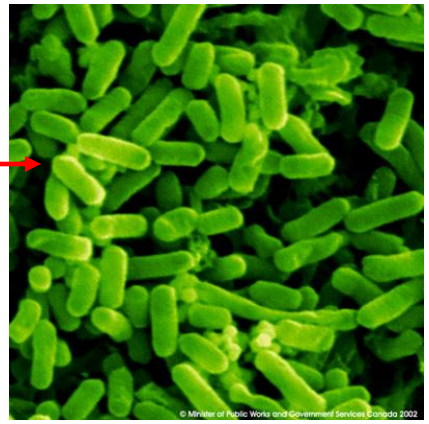
BIOHAZARD

Biosafety levels: 1.





- **Labs suitable for working with microbes that pose no risk, or a very limited risk, to lab personnel or the environment.**
- **Work is conducted on open lab bench tops.**
- **No special containment equipment is needed and foot traffic through the lab is not restricted.**
- **However, there are limited safety measures:**
  - *** contaminated work surfaces must be decontaminated**
  - *** eating and drinking is not permitted in the lab.**

BSL-1

Biosafety Level 1

Standard Microbiological Practices

Use mechanical pipetting devices

Biosafety Level 1

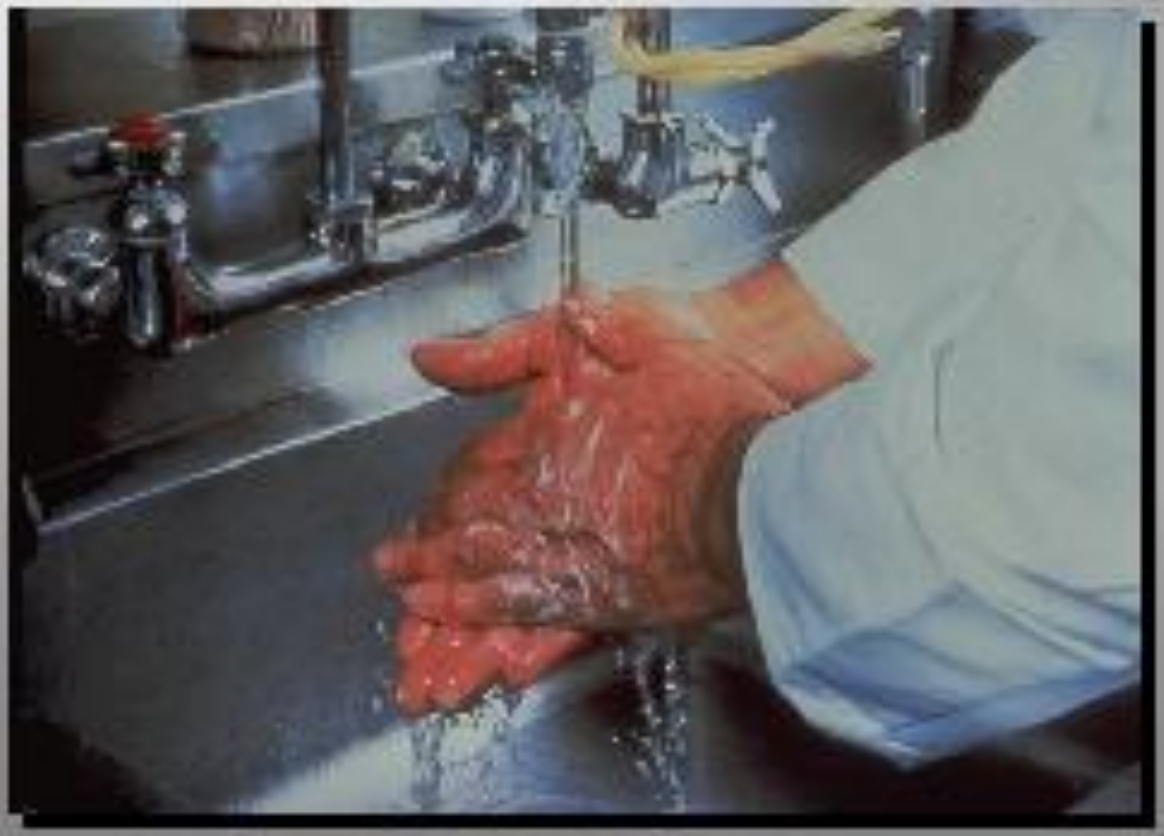
Safety Equipment (Primary Barriers)

Protective clothing

- Lab coat
- Gloves

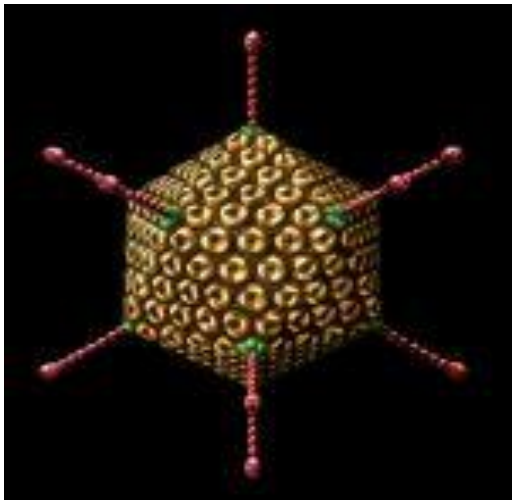
Biosafety Level 1

Standard Microbiological Practices



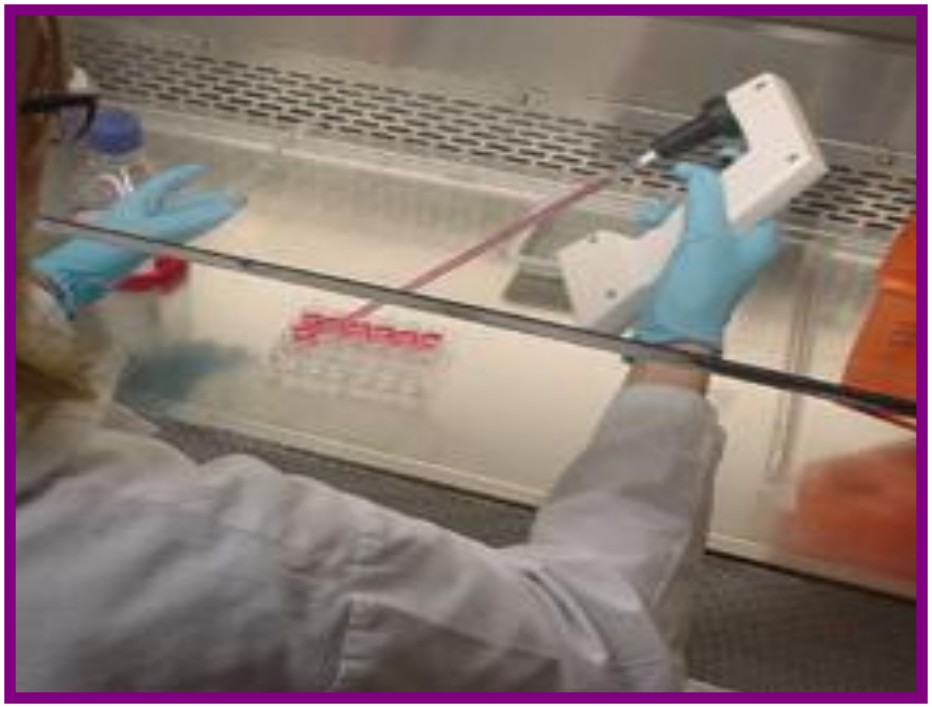
Wash hands

Biosafety levels: 2.



- **Labs are built for work with microbes of moderate risk.**
- **Access to the lab is limited when work is being conducted**
- **Lab coats must be worn,**
- **Biosafety signage is required at entrances.**
- **Special cabinets that contain an air filters are used for working with microbes.**
- **Extreme precautions are taken with contaminated sharp items**

BSL-2



Biosafety Level 2

Special Practices

Needles & Sharps Precautions

DON'T

Break, bend, resheath or reuse syringes or needles

DO

Use sharps containers

Biosafety Level 2

Special Practices (cont.)

Needles & Sharps Precautions

So someone won't be injured later

Biosafety Level 2

Special Practices (cont.)

Needles & Sharps Precautions

DON'T

Touch broken glass with hands

Biosafety levels: 3.

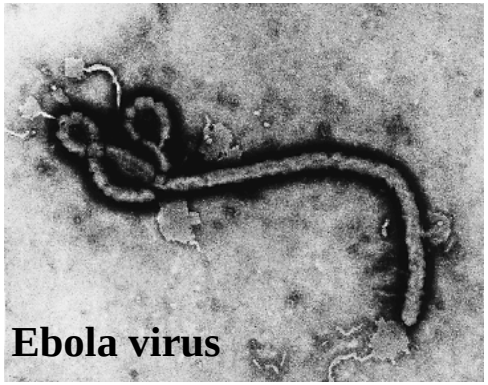
- **Labs are used for work with microbes that can cause serious injury or, in cases of an extreme exposure, death.**
- **People younger than 16 are not allowed in these labs.**
- **Lab doors must be kept closed.**
- **Windows are sealed.**


Salmonella sp.

- **All procedures are conducted within biological safety cabinets.**
- **Surgical gloves and masks, or respirators, are used.**
- **The rooms are kept at a lower air pressure level than surrounding rooms, and the outdoor environment.**
- **Air in the room also is continuously filtered through HEPA filters that are able to catch minute particles.**

BSL-3

Biosafety levels: 4.


Ebola virus

- **Labs used for work with highly infectious or dangerous microbes.**
- **Access is very restricted.**
- **Lab either in a separate building or in a controlled area within a building, completely isolated from all other areas of the building.**
- **Entry logs are kept for the labs.**
- **Lab workers must wear protective suits and undergo a disinfectant shower upon leaving.**
- **The labs have double-door airlocks.**
- **Nonbreakable containers are used for working with microbes.**
- **Materials in the lab are destroyed or decontaminated before leaving.**

BSL-4

Absolutely crucial rules

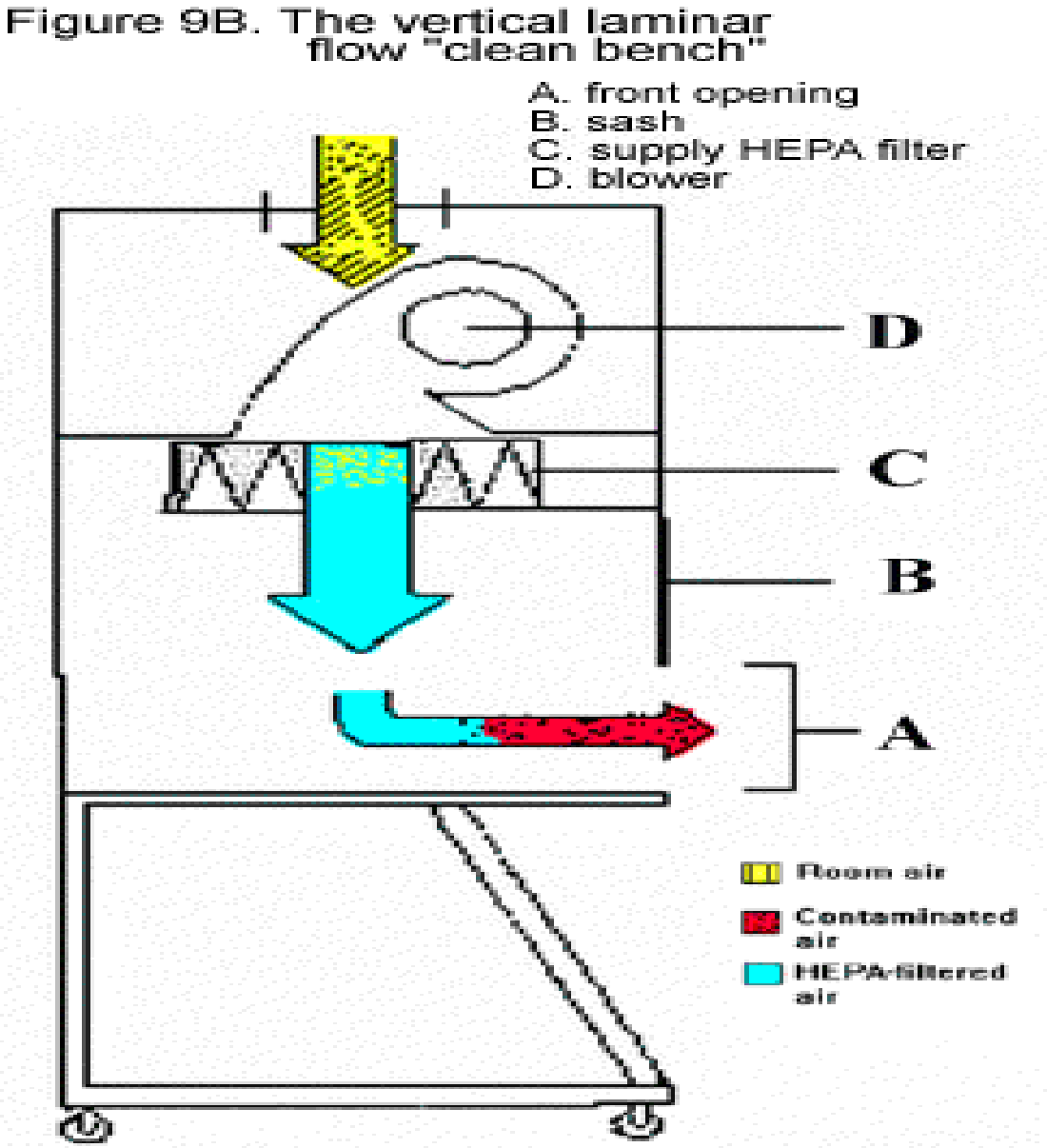
- Never smoke, drink or eat in any laboratory, because of risk of contamination by inhalation or ingestion.

- Always use the fume cupboard for hazardous chemicals.

- Always clear up at the end of session.

- Dispose of waste in appropriate containers. Most labs (including ours) have bins for sharps, glassware, hazardous solutions and radioactive waste.

Class I Biohazard hood

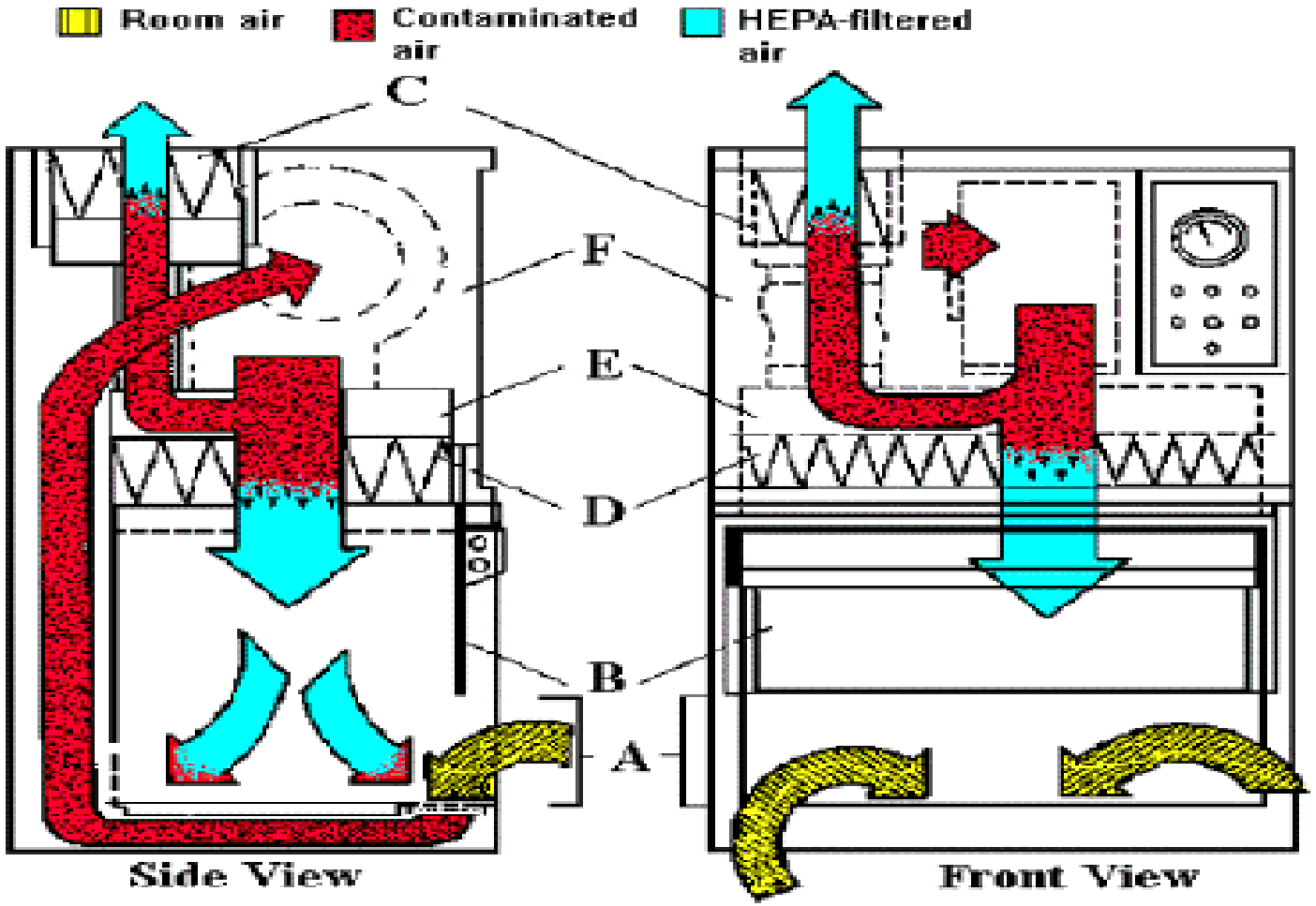


Can be used for BSL-1

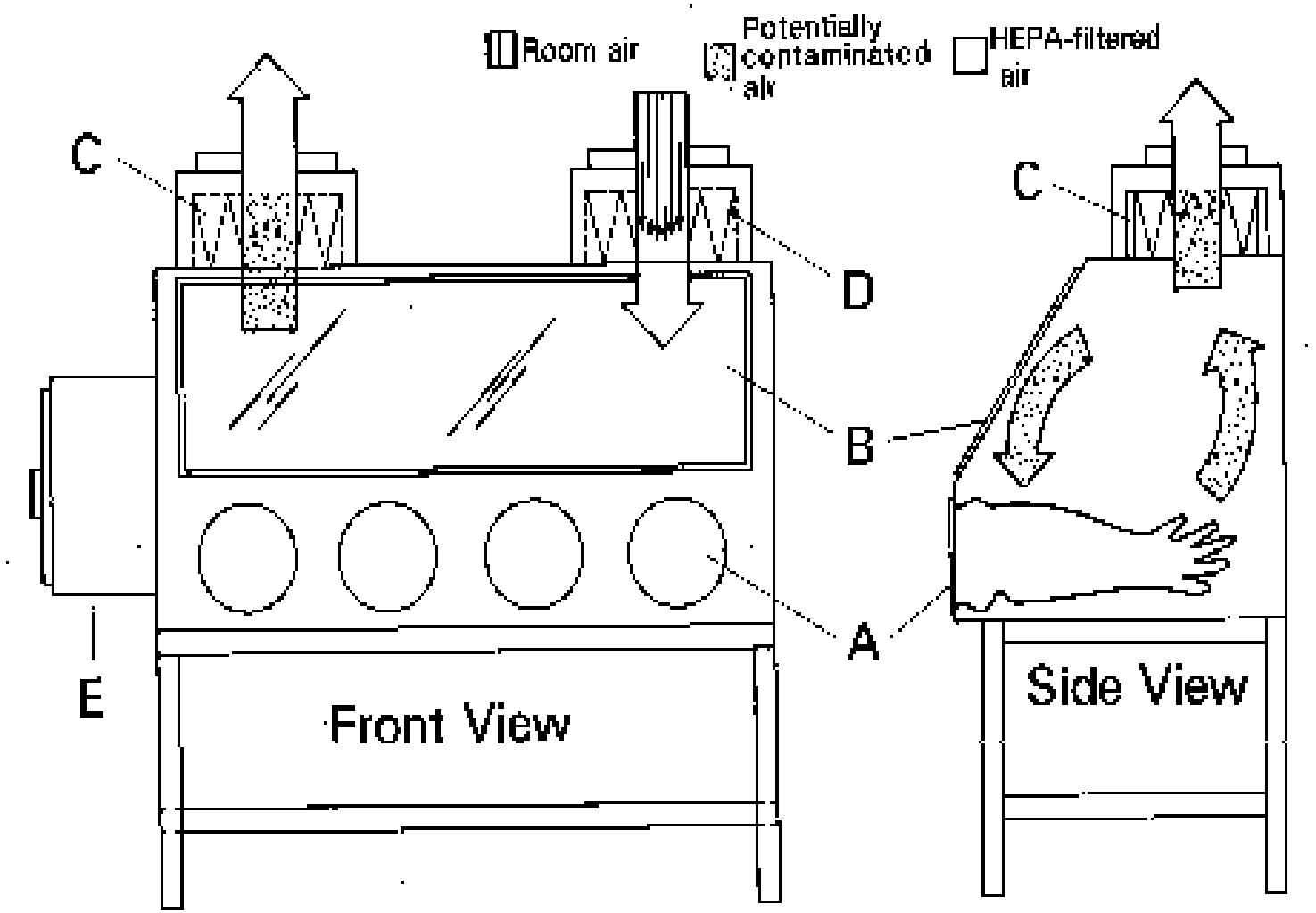
Class II Biohazard hood

Can be used for BSL-2, BSL-3, and BSL-4

A. front opening, B. sash C. exhaust HEPA filter, D. supply HEPA filter,
E. positive pressure plenum, F. negative pressure plenum
NOTE: The cabinet exhaust needs to be connected to the building exhaust system

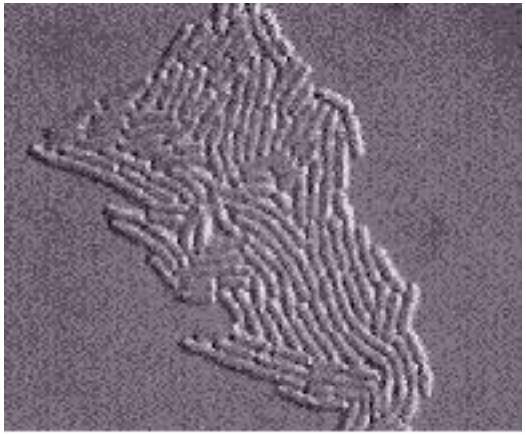


Class III Biohazard hood



Can be used for BSL-3 and BSL-4

E. coli:

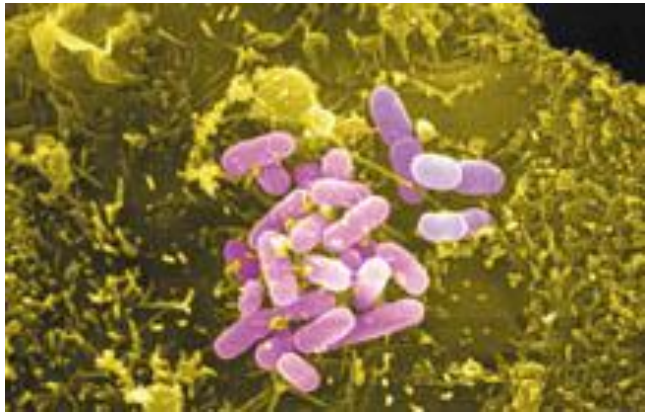


- versatile and well-adapted to its habitats

- can grow in media with glucose as the sole organic constituent

- can grow in the presence or absence of O_2. This adapts *E. coli* to its intestinal (anaerobic) and extraintestinal (aerobic or anaerobic) habitats.

E. coli:



- belongs to *Enterobacteriaceae*,

- faculatively anaerobic Gram-negative bacteria that live in the intestinal tracts of animals in health and disease

- number of genera within the family are human intestinal pathogens (e.g. *Salmonella*, *Shigella*, *Yersinia*)

- several others are normal colonists of the human gastrointestinal tract (e.g. *Escherichia*, *Enterobacter*, *Klebsiella*)

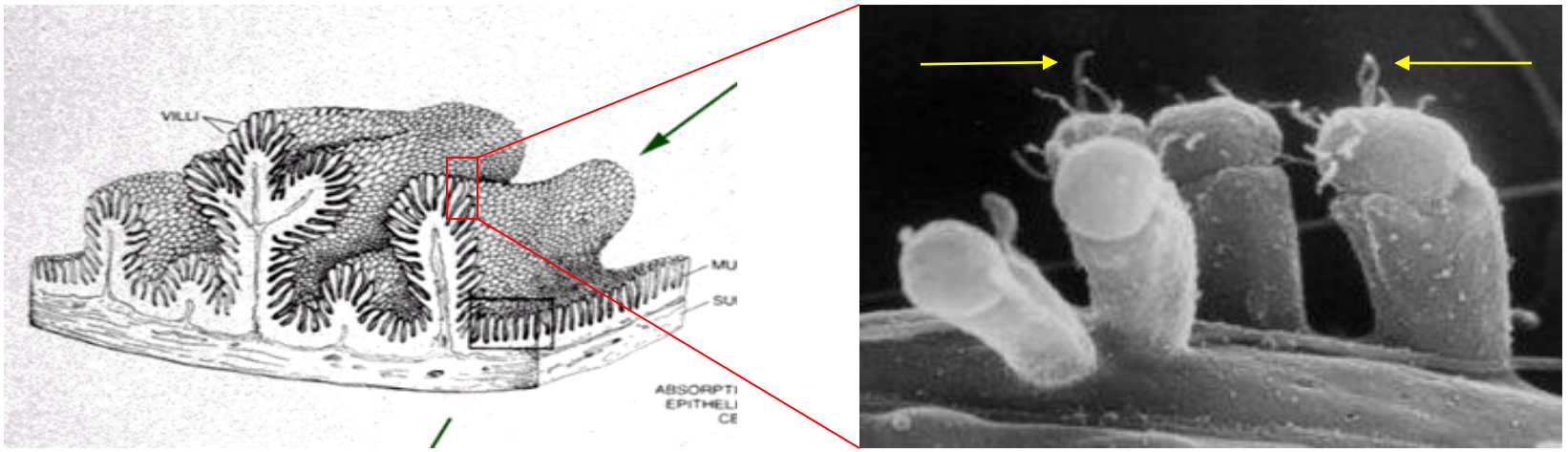
- *E. coli* is a consistent inhabitant of the human intestinal tract, however, it makes up a very small proportion of the total bacterial content. The anaerobic *Bacteroides* species in the bowel outnumber *E. coli* by at least 20:1.

E. coli:

- The gastrointestinal tract of most warm-blooded animals is colonized by *E. coli* within hours or a few days after birth.

- The bacterium is ingested in foods or water or obtained directly from other individuals handling the infant.

- The human bowel is usually colonized within 40 hours of birth. *E. coli* can adhere to the mucus overlying the large intestine.



Pathogenesis of *E. coli*

- Over 700 antigenic types (serotypes) of E. coli

- *E. coli* is responsible for three types of infections in humans:

*** urinary tract infections: *E. coli* cause 90% of the urinary tract infections in normal urinary tracts. The bacteria colonize from the feces and ascend the urinary tract to the bladder.**

*** neonatal meningitis: Neonatal meningitis affects 1 per 2,000-4,000 infants. *E. coli* strains invade the blood stream of infants from the nasopharynx or GI tract. Fortunately, invasion and the catastrophic sequelae are rare**

*** intestinal diseases (gastroenteritis): It causes diarrheal diseases in infants and travelers in underdeveloped countries or regions of poor sanitation. The diseases vary from minor discomfort to a severe cholera-like syndrome. It is acquired by ingestion of contaminated food and water.**

Strains of E. coli being used in laboratory practice are not pathogenic.

Selection of the proper bacterial strain

- **For inducible expression of recombinant proteins, a strain that carries an inducible T7 RNA Polymerase gene are useful, especially for proteins which are toxic to *E. coli*.**

- **For mutagenesis experiments, bacteria deficient in mismatch repair mechanisms are utilized to allow replication of the mutated daughter strand (i.e. *mutS* strains).**

- **To propogate M13 and other single-stranded DNA bacteriophages, bacteria must possess a sex pili encoded by an F factor.**

- **For expression of recombinant or fusion proteins, a strain of bacteria that is defective in protease activity may be beneficial (i.e., *lon* mutation should be resent).**

- **Bacterial strain should not carry endogenous resistance to the antibiotic *($AmpR^-$)* resistance gene present on the plasmid of interest.**

- **If blue/white screening is going to be employed, the host cell strain should possess an episomal and/or chromosomal deletion of the alpha-peptide coding region for β-galactosidase (i.e., *LacZ*δM15).**

- **A strain that is deficient in recombination mechanisms *($RecA^-$)* may be selected if recombination between the insert and vector or chromosomal sequences is problematic.**

- **Increased yield of DNA due to endA mutation (decreased endonuclease activity)**

Examples of bacterial strains

JM109

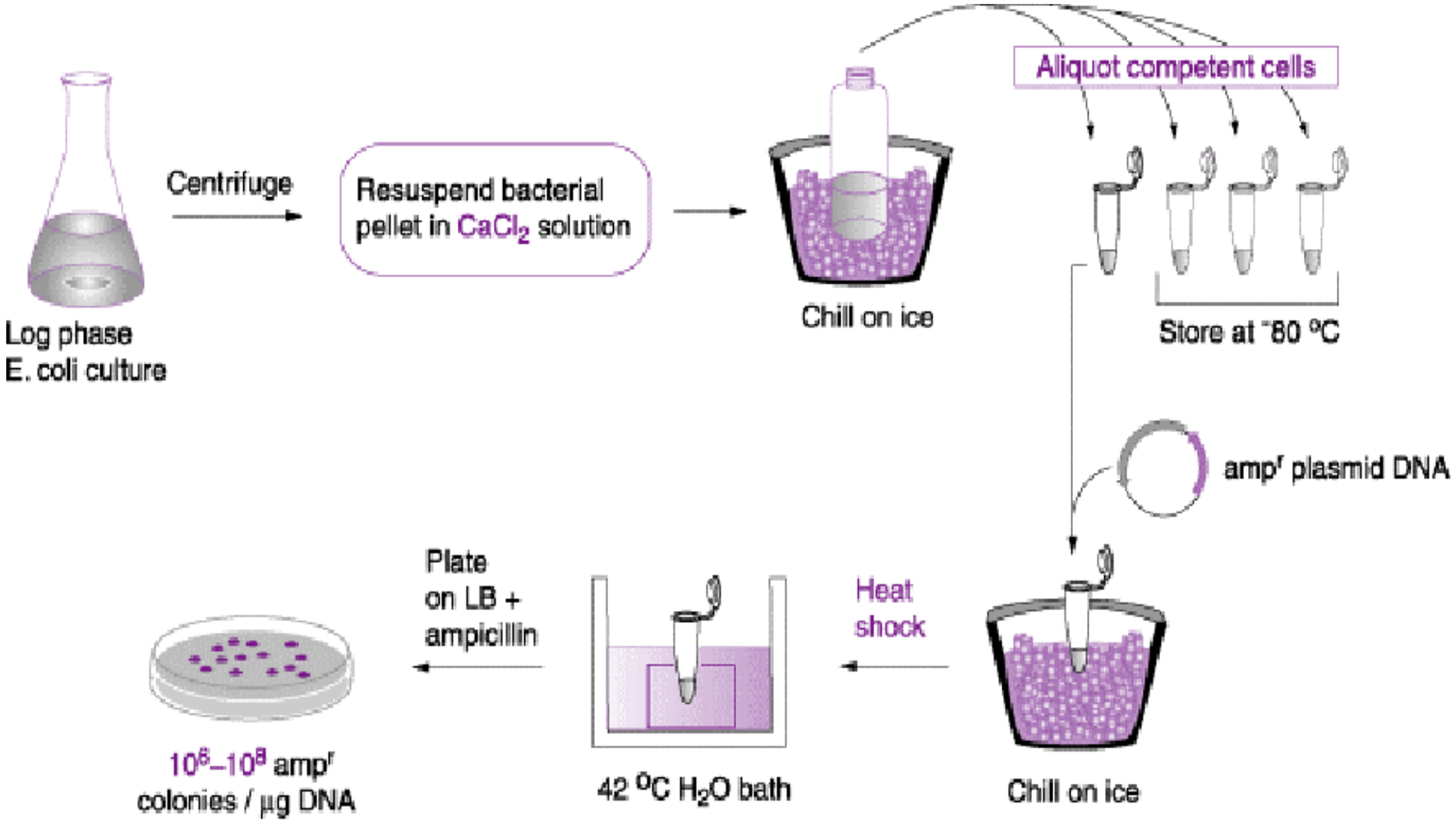
- carries *recA1* (decreased recombination rate) and *endA1* (decreased endonuclease-1 activity) mutations that improves plasmid stability, quality and yields
- *(r- m-)* genotype (non-methylated plasmid is not digested)
- Blue/white color selection
- Single-strand propagation from M13 or phagemid vectors
- Very good for routine sub-cloning

Can be used for subcloning into pBS and pShuttle2.

DH5α or XL10-Gold

- plasmid stability due to to *recA1* mutation
- blue/white screening
- DH5α accept large plasmids due to *deoR* mutation (a mutation in this gene allows constitutive synthesis for genes involved in deoxyribose synthesis); in XL-Gold mutation is not characterized
- high plasmid yield due to *endA1* mutation

Can be used for subcloning into pAdeno.


Log phase
E. coli culture
Centrifuge
Resuspend bacterial pellet in $CaCl_2$ solution
Chill on ice
Aliquot competent cells
Store at −80 °C
amp^r plasmid DNA
Chill on ice
Heat shock
42 °C H_2O bath
Plate on LB + ampicillin
10^6–10^8 amp^r colonies / µg DNA

Transformation of bacteria

- Do not expose competent bacteria to room temperature

At no time the cells should be exposed to room temperature except for few seconds. Gently mix the cells by tapping with your fingers, and NOT by pipetting. Then shake the tube down in one single motion from elbow, so the mixture will be collected. Place it on ice.

- Pre-warm Agar Plates

Cold plates dramatically decreased the transformation efficiency. It is strongly recommended that agar plates are prewarmed at 37°C or at least prewarmed over 20°C.

- Incubation Time

For optimum transformation, incubation on ice should take 10 to 20 minutes; longer incubation up to 50-60 minutes is okay too. Extended incubation over 2-4 hours reduces the transformation efficiency gradually.

-Out Growth (Addition of SOC)

By adding of SOC (e.g. 400 µl of SOC to 100 µl of transformation mixture) after incubation on ice and incubating for 1 hour at 37°C the transformation efficiency can be increased by 2-3 fold. This step is **not necessary for ampicillin resistance**. When kanamycin, tetracycline, chloramphenicol, erythromycin and any non-lactamase selection markers are used, an out growth step is needed for efficient transformation. Reducing reagents, such as DTT and 2-mercaptoethanol, are not needed for this procedure (but they can increase efficacy).

Antibiotic	**Working Concentration**	**Stock Concentration**
Ampicillin (Amp)	50-100µg/ml	50mg/ml in water
Chloramphenicol (Cm)	20-170µg/ml	34mg/ml in ethanol
Kanamycin (Kan)	30µg/ml	50mg/ml in water
Streptomycin (Sm)	30µg/ml	50mg/ml in water
Tetracycline (Tet)	10µg/ml liquid culture; 12.5µg/ml plates	12.5mg/ml in ethanol