Clinical Microbial Laboratory Investigation

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Biofilm Interest Group | BiG
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Biofilm in clinical samples

- Microorganisms universally attach to surfaces and produce extracellular polysaccharides, resulting in the formation of a biofilm.
- Biofilms pose a serious problem for public health because of the increased resistance of biofilm-associated organisms to antimicrobial agents and the potential for these organisms to cause infections in patients with indwelling medical devices.
- An appreciation of the role of biofilms in infection should enhance the clinical decision-making process.
- Many bloodstream infections and urinary tract infections are associated with indwelling medical devices and, therefore, are (in most cases) biofilm associated.
- Conventional treatments with antibiotics and steroids are often useless .
- At the moment, the most effective strategy for treating these infections may be removal of the biofilm contaminated device.

Biofilms on medical devices

- Prosthetic heart valves
- Central venous catheters
- Urinary catheters
- Contact lenses
- Endotracheal tubes
- Intrauterine devices
- Mechanical heart valves
- Pacemakers
- Peritoneal dialysis catheters
- Prosthetic joints
- Tympanostomy tubes
- Urinary catheters
- Dental unit water lines

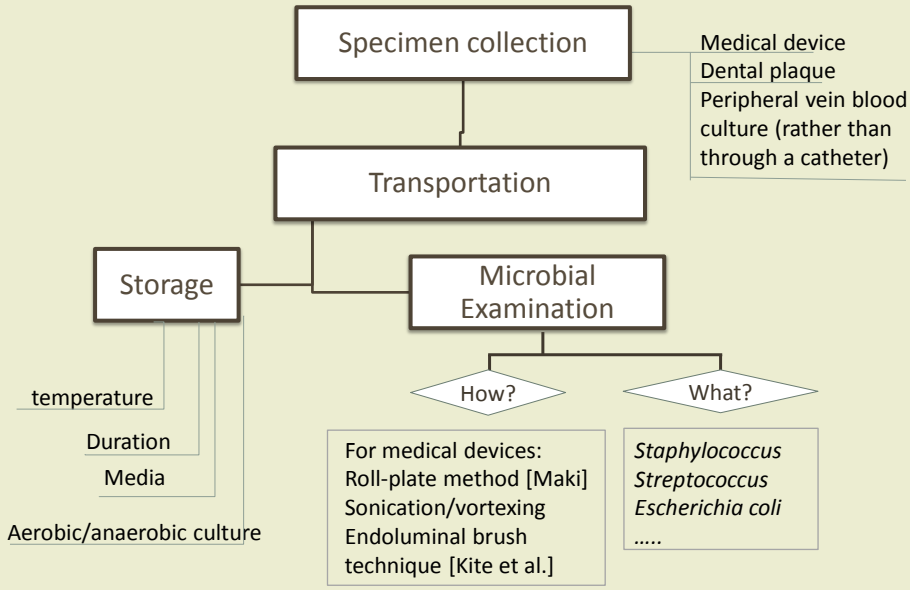
Biofilms in medical devices

- Composed of gram-positive or gram-negative bacteria or yeasts:
  - gram positive *Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis,* and *Streptococcus viridans*;
  - gram-negative *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis,* and *Pseudomonas aeruginosa*.
  - These organisms may originate from the skin of patients or healthcare workers, tap water to which entry ports are exposed, or other sources in the environment.
- Pure culture or poly-microbial, depending on the device and its duration of use in the patient.

Table 2. Biofilm-associated microorganisms commonly isolated from selected indwelling medical devices.

Indwelling medical device	Organisms
Central venous catheter	Coagulase-negative staphylococci, *Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans*
Prosthetic heart valve	Viridans *Streptococcus*, coagulase-negative staphylococci, enterococci, *Staphylococcus aureus*
Urinary catheter	*Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Proteus mirabilis*
Artificial hip prosthesis	Coagulase-negative staphylococci, β-hemolytic streptococci, enterococci, *Proteus mirabilis, Bacteroides* species, *Staphylococcus aureus*, viridans *Streptococcus, Escherichia coli, Pseudomonas aeruginosa*
Artificial voice prosthesis	*Candida albicans, Streptococcus mitis, Streptococcus salivarius, Rothia dentrocariosa, Candida tropicalis, Streptococcus sobrinus, Staphylococcus epidermidis, Stomatococcus mucilaginous*
Intrauterine device	*Staphylococcus epidermidis, Corynebacterium* species, *Staphylococcus aureus, Micrococcus* species, *Lactobacillus plantarum*, group B streptococci, *Enterococcus* species, *Candida albicans*

Important considerations when taking specimens



General considerations to collect specimen

1. Whenever possible, specimens should be obtained **before antibiotics** or other antimicrobial agents have been administered.
2. Clinical material should be collected **in leak-proof** specimen **containers** that are tightly sealed.
3. Material should be collected where the suspected organism is most likely to be found and with as little external **contamination** as possible (this is particularly important for draining lesions).
4. Specimens should be of **sufficient quantity** to permit completion of all tests ordered.
5. Provisions should be made for the **prompt delivery** (**within one hour** after collection) of the specimen to the laboratory.
6. Most clinical material can be held for several hours in a refrigerator (NOT freezer).

Diagram of an intravenous catheter with biofilm growth.

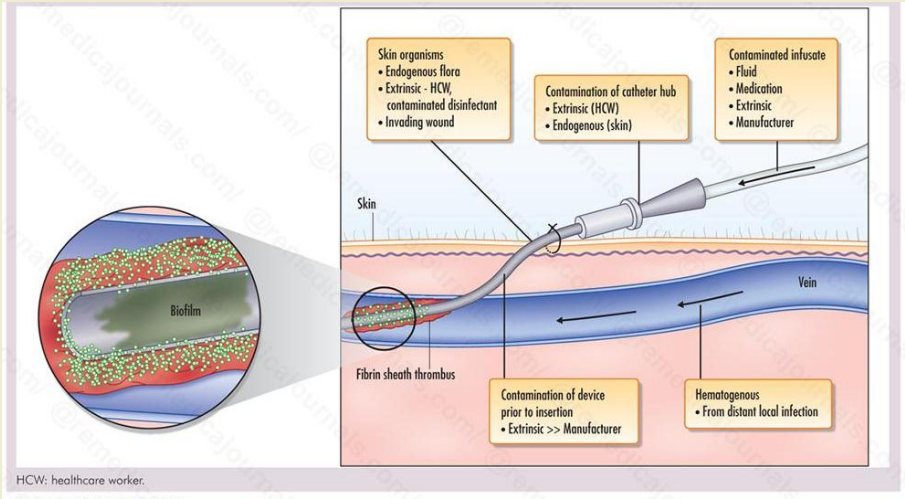
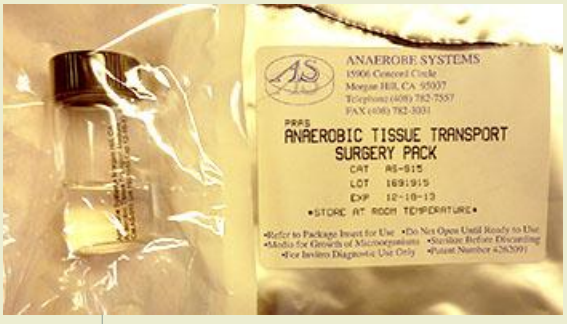


Image courtesy of Remedica Journals
http://www.remedicajournals.com/The-Journal-of-Invasive-Fungal-Infections/BrowseIssues/Volume-5-Issue-2/Article-The-Story-of-Biofilms



Anaerobic media for tissue

Blood culture bottles

Protocol: Blood collection

- In most adult infections, cultures of two to three separate venipunctures (20-30 mL per venipuncture) are sufficient.
- Take time to properly disinfect the draw site because contaminated cultures may provoke unnecessary treatment or procedures for some patients.
- Drawing blood for culture from catheters is not an outpatient procedure and may be performed only by authorized staff.

Protocol: Wound collection

- Tissue or aspirates are always superior to swab specimens.
- Tissue taken from the base or leading edge of the wound is more likely to recover viable clinically significant organisms than sampling of necrotic material.

1. Remove surface exudate by wiping with sterile saline or 70% alcohol.
2. Aspirate with needle and syringe.
3. Cleanse rubber stopper of anaerobic transport vial with alcohol; allow to dry 1 min before inoculating; push needle through septum and inject all abscess material on top of agar.
4. If a swab must be used, pass the swab deep into the base of the lesion to firmly sample the fresh border.
5. Transport time $\leq$ 2 hours.

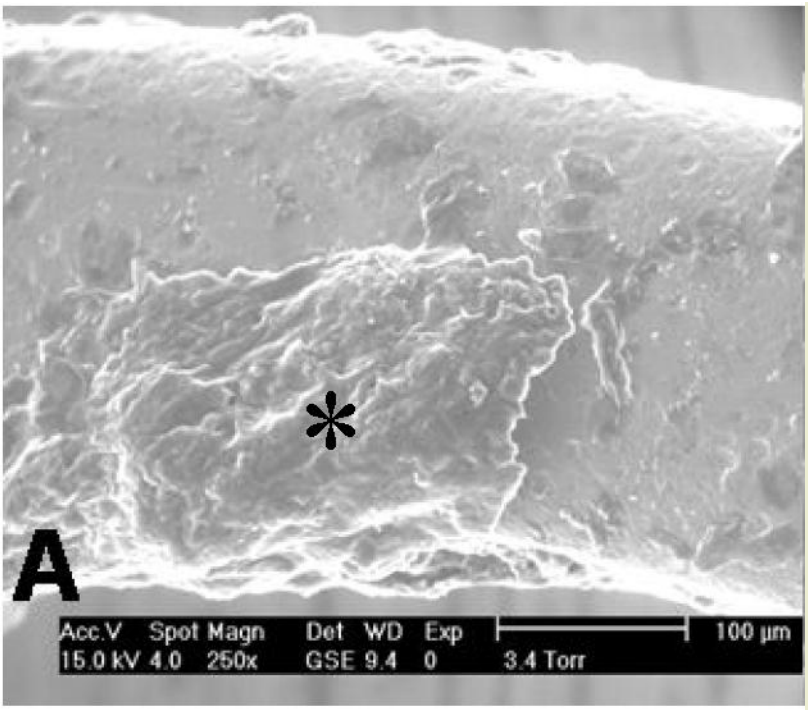
Biofilm examination

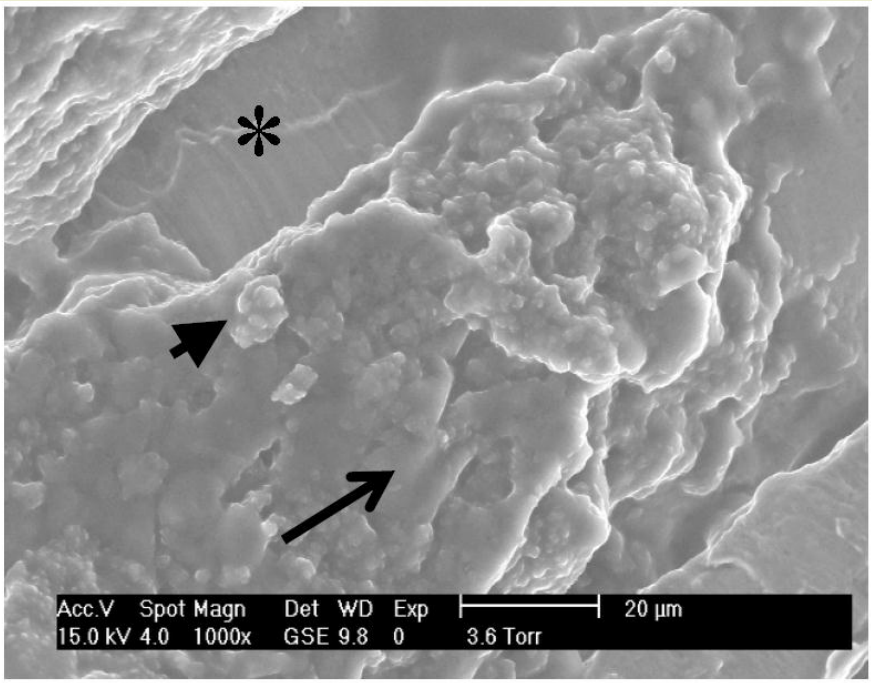
- Electron microscopy technique :
  - utilizes graded solvents (alcohol, acetone, and xylene) to gradually dehydrate the specimen prior to examination, since water of hydration is not compatible with the vacuum used with the electron beam.
  - results in significant sample distortion and artifacts; the extracellular polymeric substances, which are approximately 95% water, will appear more as fibers than as a thick gelatinous matrix surrounding the cells.
  - transmission electron microscopy and specific polysaccharide stains like **ruthenium red** allowed researchers both to identify the nature of these extracellular fibers in biofilms and to better elucidate their association with the cells.
- Confocal laser scanning microscopy
- Vortexing or sonication:
  - more common
  - remove biofilms or biofilm-associated organisms from the substratum by some type of mechanical force
  - Followed by viable plate count procedure, in which the resuspended and dispersed biofilm cells are plated onto a solid microbiological medium, incubated, and counted.

General considerations to transport specimen

- **OTHER STERILE BODY FLUID**
- Follow standard procedures and obtain the specimen by **aspiration**.
- Transport the specimen in **aerobic or anaerobic transport kits** or blood culture bottles depending on clinical condition. Specimens may be submitted in sterile containers for aerobic culture only.

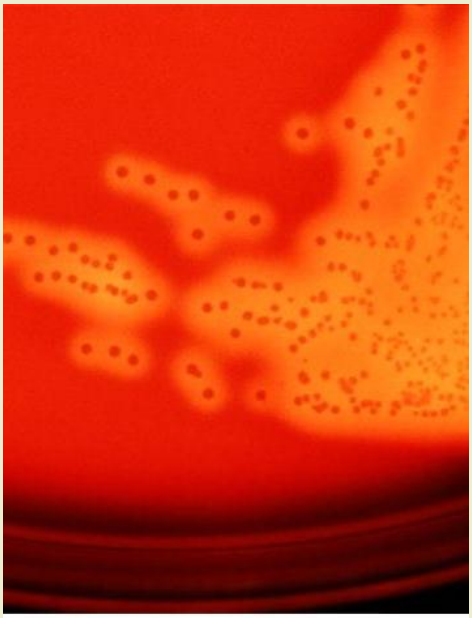
- **TISSUE BIOPSY SAMPLE**
- Submit 1 gram of tissue, if possible, in a sterile container without fixative or preservative. Keep moist with a small quantity of **sterile saline** or **nutritive broth**.
- Collect aseptically and avoid indigenous microbiota. Select caseous portion if available. Refrigerate. Do not freeze.
- Store and transport specimens at 4°C.





Blood agar plate showing:
(a) small colony variant;
(b) normal variant of *Staphylococcus aureus*

Rit K. A case report of Small Colony variant of *Staphylococcus aureus* isolated from a patient with chronic oesteomyelitis in a tertiary care hospital of eastern India. Adv Biomed Res 2014;3:32




That's all – Thank you