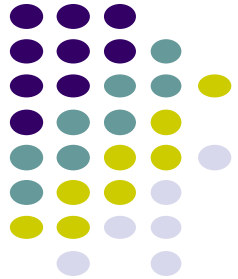
بسم الله الرحمن ارحیم

نمونه گیری در میکروب شناسی

نقش آزمایشگاه میکروب شناسی در درمان بیمار

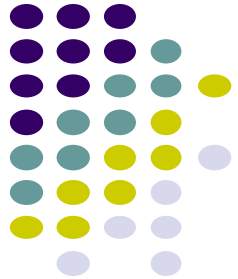
- تجزیه و مدیریت نمونه ها
- ارتباط موثر با افرادی که در درمان بیمار درگیرند ، قبل و بعد از تجزیه نمونه.

Summarize of Microbiology Laboratory Procedures



1. Specimen collection
2. Observation
3. Isolation
4. Identification
5. Antimicrobial Susceptibility Test

Specimen Selection

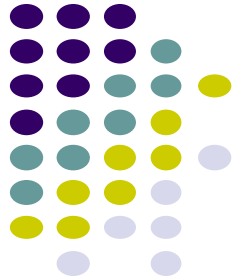


- *The specimen must be material from actual infection site and must be collected with a minimum of contamination from adjacent The specimen must be material from actual tissues ,organs, or secretions.*

- *Optimal times for specimen collection must be established for the best chance of recovery of causative microorganisms.*

- *A sufficient quantity of specimen must be obtained to perform the culture techniques requested.*

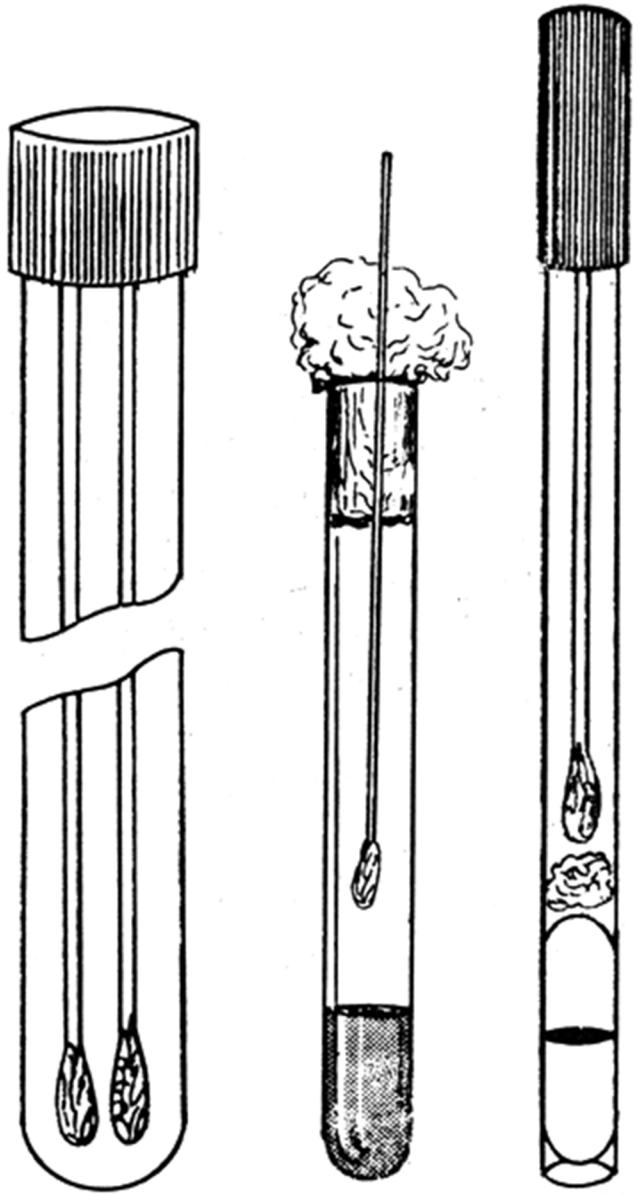
Specimen Collection

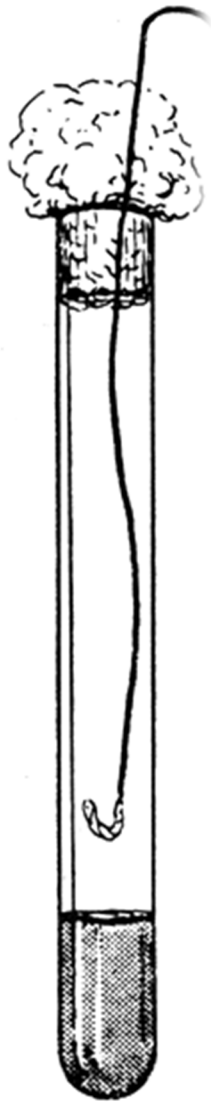


- *Appropriate collection devices, specimen containers, and culture media must be used to ensure optimal recovery of microorganisms.*
- *Culture must be obtained prior to the administration of antibiotics.*
- *The culture container must be properly labeled.*
- *Provide complete information on specimen requisition forms.*

Specimen Transport Systems

- *Sterile screw-cap cups, Petri dishes, tubes*
- *Swabs*
  - *Swab Transport system*
    - *In order of fiber material*
      - *Cotton Swabs*
      - *Calcium Alginate Swabs*
      - *Dacron Swabs*
      - *Rayon Swabs*
  - *Nasopharyngeal-urethrogenital Swabs*
  - *N.gonorrhoeae transport systems*
  - *Proprietary swab systems for molecular testing for GC/CT*
  - *Anaerobic Transport Systems*
  - *Viral Transport Systems*





شکل ۳ – ۲ سوابهای مختلف

شکل ۳ – ۲


Sterillin

Specimen Transport Systems

- *Sterile screw-cap cups, Petri dishes, tubes*
- *Swabs*
  - *Swab Transport system*
    - *In order of fiber material*
      - *Cotton Swabs*
      - *Calcium Alginate Swabs*
      - *Dacron Swabs*
      - *Rayon Swabs*
  - *Nasopharyngeal-urethrogenital Swabs*
  - *N.gonorrhoeae transport systems*
  - *Proprietary swab systems for molecular testing for GC/CT*
  - *Anaerobic Transport Systems*
  - *Viral Transport Systems*

Canterbury Health Laboratories

SWAB IDENTIFICATION GUIDE

BACTERIAL SWABS

Phone Microbiology, ext 80350

Blue top Bacterial swab with Transport Medium (not for PCR)

ALL BACTERIAL SWABS
including:
HIGH VAGINAL SWAB
for Gram stain,
routine culture, yeasts,
Trichomonas vaginalis
CERVICAL SWAB
for *N. gonorrhoeae*,
Mycoplasma, Ureaplasma

CHLAMYDIA SWABS

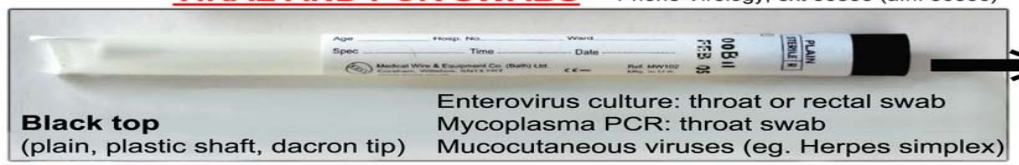
Order from Serology, ext 80416 (a.h. 80350)

Pink top Chlamydia swab FOR CERVIX ONLY

Blue top Chlamydia swab FOR URETHRA OR EYES

VIRAL AND PCR SWABS

Phone Virology, ext 80356 (a.h. 80350)



Black top
(plain, plastic shaft, dacron tip)

Enterovirus culture: throat or rectal swab
Mycoplasma PCR: throat swab
Mucocutaneous viruses (eg. Herpes simplex)

Viral Transport medium (VTM)
not suitable for bacterial culture
as it contains antibiotics

Respiratory viruses: nasopharyngeal swab

Orange top (pernasal swab) **FOR ADULTS**

Green stripe (flocked swab) **FOR NEONATALS**

Viral Transport medium (VTM)
not suitable for bacterial culture
as it contains antibiotics

***Bordetella pertussis* PCR:** nasopharyngeal swab>> place back into collection tube

Orange top (pernasal swab) **FOR ADULTS**

Green stripe (flocked swab) **FOR NEONATALS**

Viral Transport medium (VTM)

Microbiology Department, Canterbury Health Laboratories, June 2007

Group A Strep Swab

Use for molecular probe-group A Strep or group A Strep Rapid Screen.

• DO NOT USE FOR CULTURE •

• USE FOR THROAT SPECIMENS ONLY •

Molecular Probe Group A Group A Strep Rapid Screen

General Culture Swab

Use for bacterial and fungal culture.

A separate swab is required for each test type.

Cystic Fibrosis culture	Gram Stain
Fungal culture	Routine Culture
Molecular Probe Group B Strep	
Rapid viral antigen*	Viral culture*

*Place swab in ***Viral Transport Media (VTM).***

Charcoal Swab

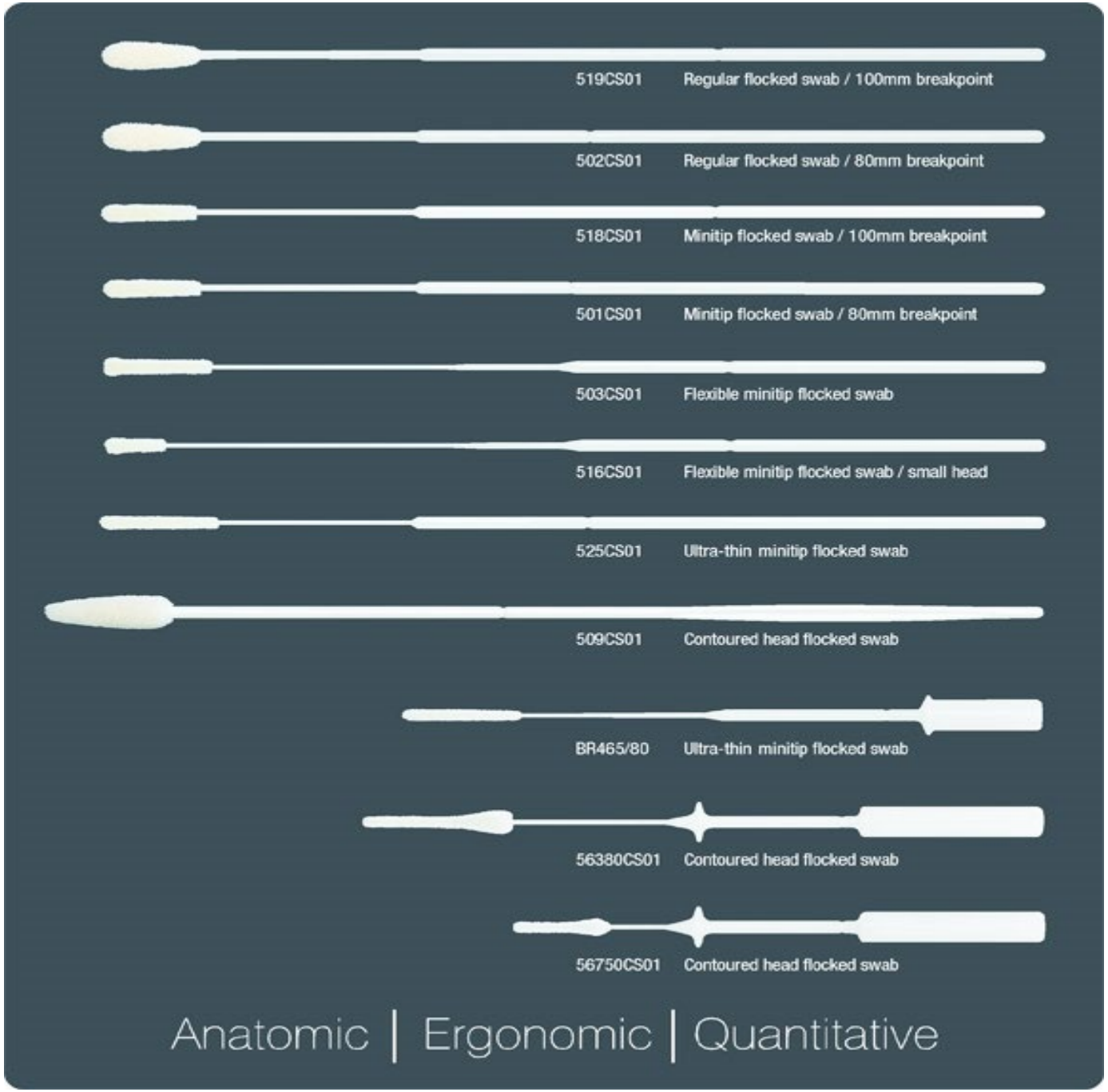
Use for culture of Neisseria gonorrhoeae (G. C.) on specimens collected from rectal and throat sources. Recommended for ***all*** specimen types when sexual assault or abuse are under consideration as well as for specimens from prepubescent females (<14 y.o.).

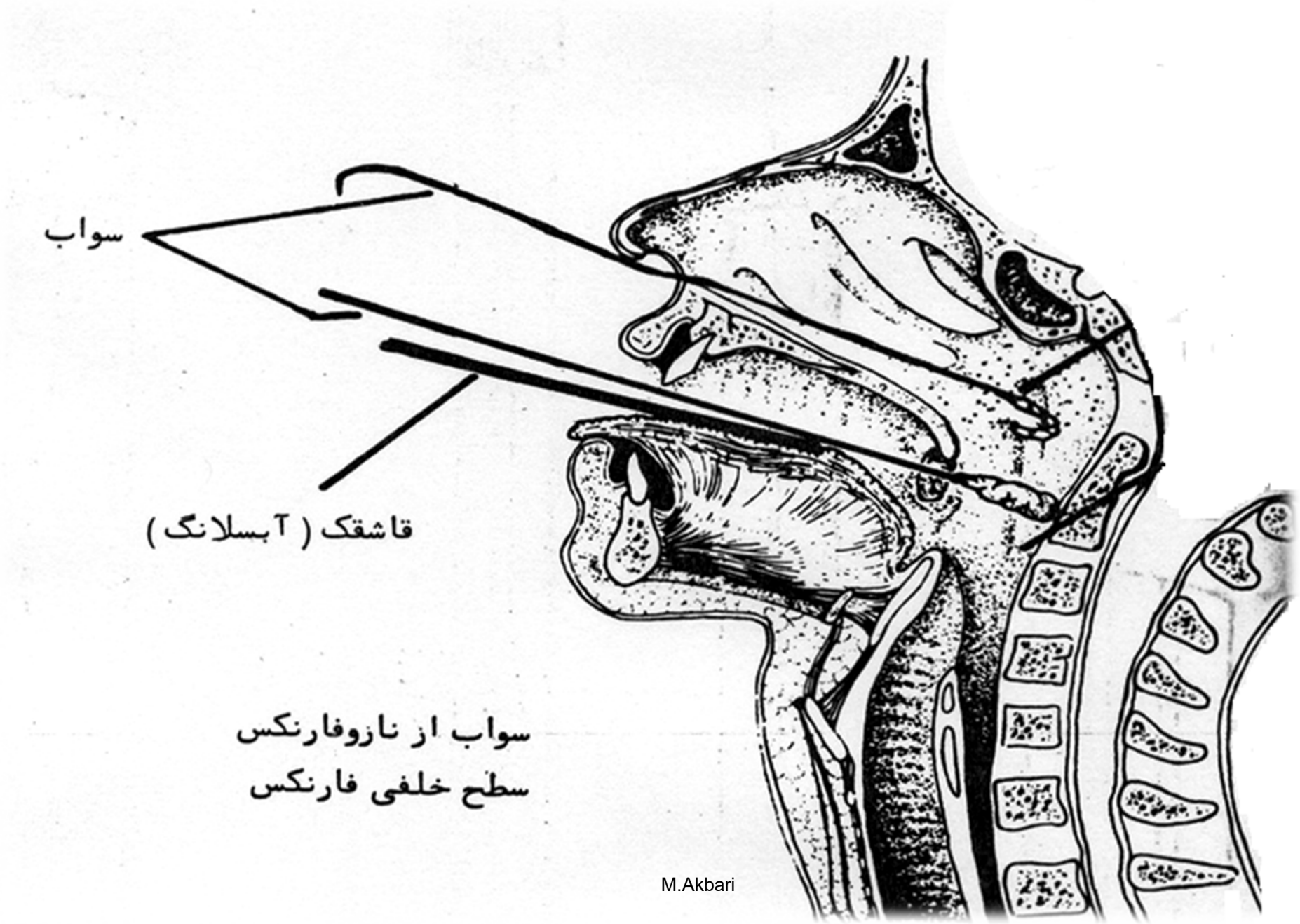
Culture – G. C.

Minitip Swab

Use for culture collected from nasopharynx or male urethra.

Bordetella culture and DFA	(place in Jones-Kendrick media-see next column)
Rapid viral antigen	(place in VTM – see next column)
Viral culture	(place in VTM – see next column)
G. C. culture	(place in charcoal swab holder for transport)

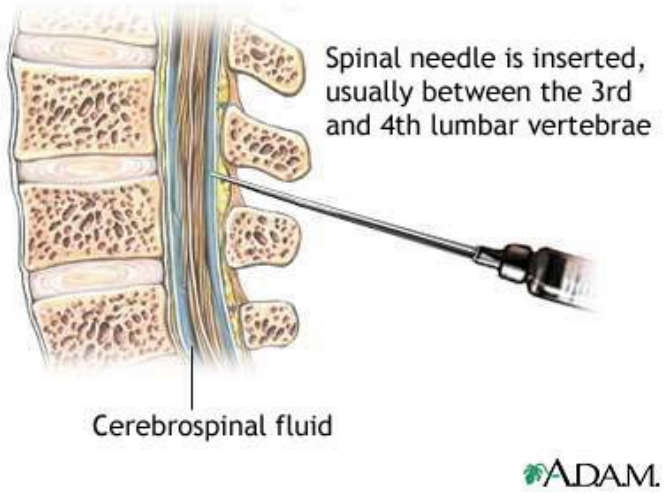

519CS01 Regular flocked swab / 100mm breakpoint
502CS01 Regular flocked swab / 80mm breakpoint
518CS01 Minitip flocked swab / 100mm breakpoint
501CS01 Minitip flocked swab / 80mm breakpoint
503CS01 Flexible minitip flocked swab
516CS01 Flexible minitip flocked swab / small head
525CS01 Ultra-thin minitip flocked swab
509CS01 Contoured head flocked swab
BR465/80 Ultra-thin minitip flocked swab
56380CS01 Contoured head flocked swab
56750CS01 Contoured head flocked swab
Anatomic | Ergonomic | Quantitative


سواب
قاشقک (آبسلانگ)
سواب از نازوفارنکس
سطح خلفی فارنکس

ودرنهایت سوالات کلیدی

۱. **از کجا** نمونه مناسب باید گرفته شود؟
۲. **چه کسی** باید نمونه گیری را انجام دهد؟
۳. **چه وقتی** زمان مناسب نمونه گیری
۴. **چه مقدار** نمونه باید جمع آوری شود؟
۵. **روش نمونه گیری** مناسب برای این نمونه چه روشی است؟
۶. **وسایل مورد نیاز** برای نمونه گیری چه هستند (بر اساس روش)؟
۷. **چه تعداد** نمونه گرفته شود؟
۸. **فاصله زمانی** بین نمونه ها چقدر باشد؟
۹. **نحوه انتقال** نمونه و **تمهیدات لازم** (دما، اتمسفر و ...) جهت انتقال نمونه به آزمایشگاه چه هستند؟

CSF Collection



CSF Collection and Processing

Major Pitfalls and Controversies

- Failure to properly decontaminate
- VOLUME
- Timely transport
- Pre-treatment with antibiotics
- Bacterial antigen detection tests
- Media
- Duration of incubation

Inoculation of Sterile Body Fluids to Blood Culture Bottles

- Inoculate blood culture bottles at bedside when volume is sufficient
  - Minimum of 1 mL of specimen/bottle
  - Collect aerobic and anaerobic bottle set
  - BacT/Alert FAN bottles outperformed conventional media and standard bottles in several studies
- Send additional fluid in sterile container for immediate Gram's stain and culture for Mycobacteria, fungus or "special" pathogens
- Most useful for synovial fluids and peritoneal fluid/CAPD; literature mixed for pleural fluid

Wounds:Classification

Acute

- Caused by external damage to intact skin
- Types
  - Surgical
  - Bites
  - Burns
  - Minor cuts
  - Abrasions
  - Severe traumatic

Chronic

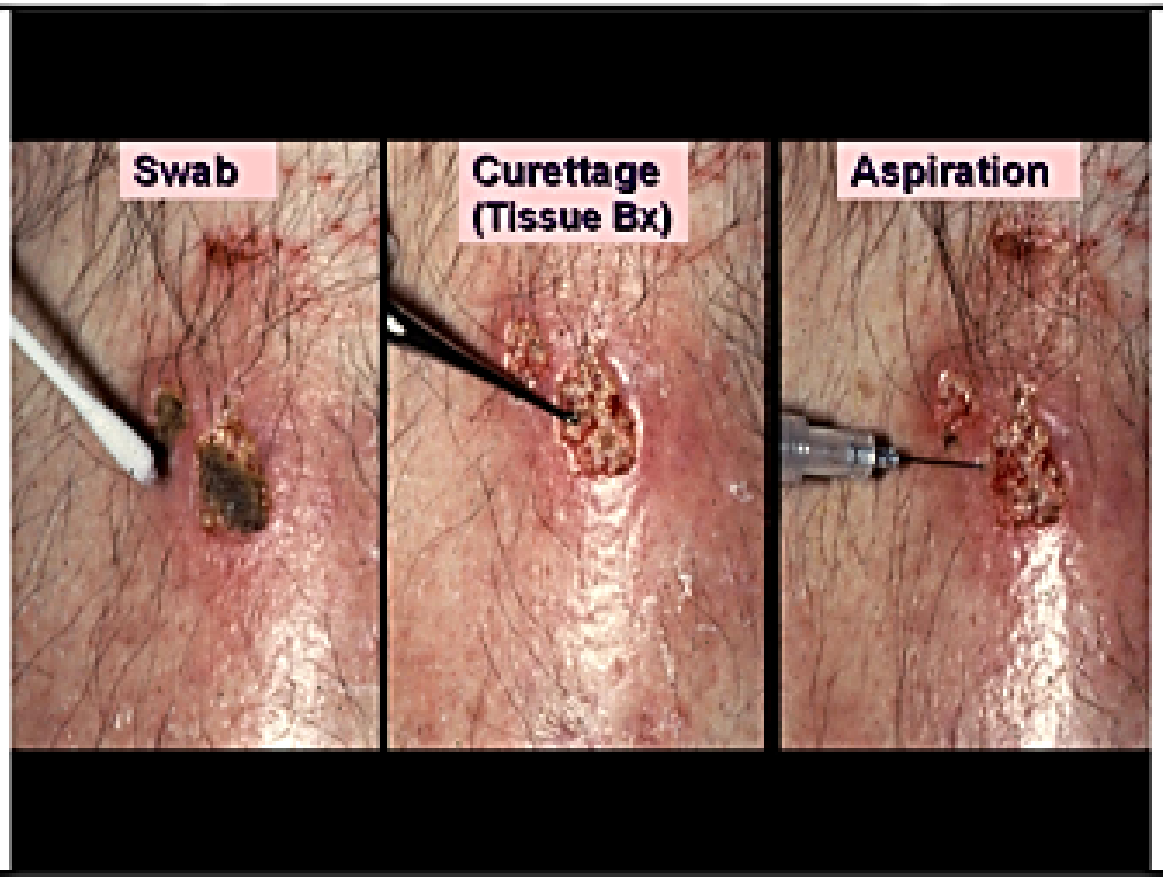
- Precipitated by predisposing conditions that lead to compromise of dermal/epidermal tissue
- Types
  - Impaired venous drainage
  - Impaired arterial supply
  - Metabolic diseases eg. diabetes

Bowler PG, et. al. 2001. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* 14: 244.

Wound Cultures

- For open wounds
  - Clean the wound margins with surgical soap or 70% ethyl or isopropyl alcohol
  - Aspirate from the depth of the wound using a sterile syringe and needle
  - Aspirated fluid should be sent to the laboratory in an appropriate transport system
  - Alternatively, a curette may be used to obtain tissue from base of the wound
  - Swabs are strongly discouraged

X. Proper methods for culturing wounds

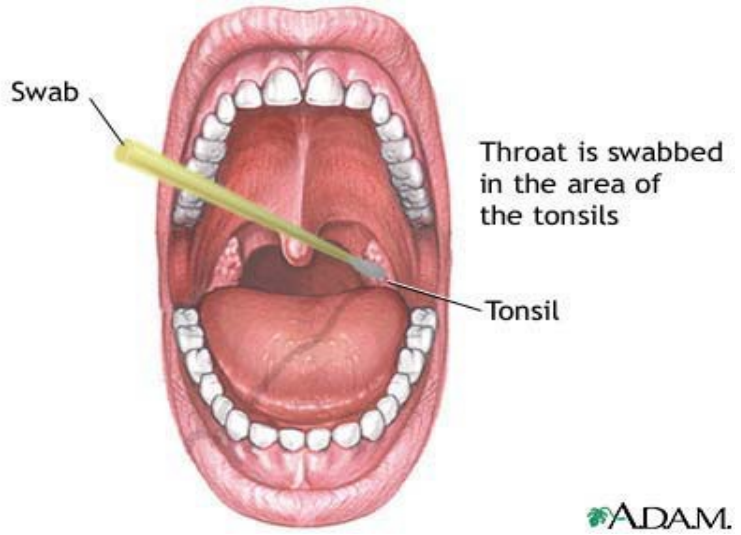


Wound Cultures

- For closed wounds
  - Prepare site as described for obtaining blood culture
  - Aspirate as much purulent material as possible
  - Transport in aerobic/anaerobic transport system

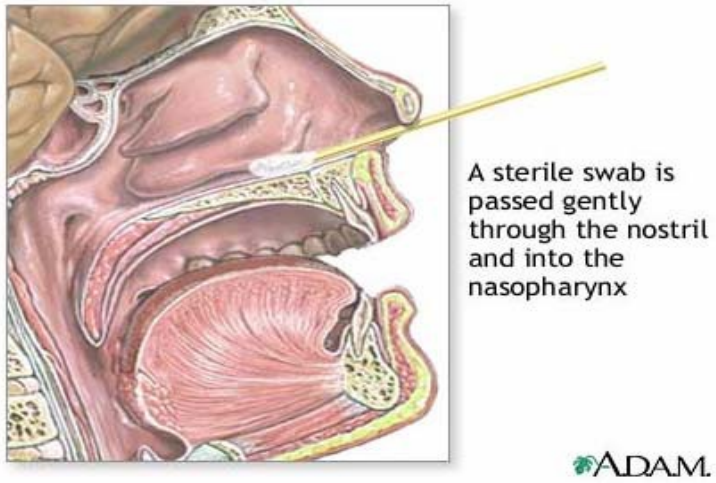
Respiratory Specimens

- Upper respiratory tract specimens
  - Throat
    - detection of streptococcal pharyngitis



Respiratory Specimens

- Upper respiratory tract specimens
  - Nose
    - detection of MRSA carriers
  - Nasopharyngeal swabs
    - diagnosis of *Bordetella pertussis*
  - Nasopharyngeal swabs and washings
    - diagnosis of viral disease



Sputum Collection

- Proper patient instruction
  - Food should not have been ingested for 1-2 h prior to expectoration
  - The mouth should be rinsed with saline or water
  - Patient should breathe and cough deeply
  - Patient should expectorate into a sterile container
- Transport container immediately to lab
- Perform Gram stain and plant specimen as soon as possible

Sputum collection

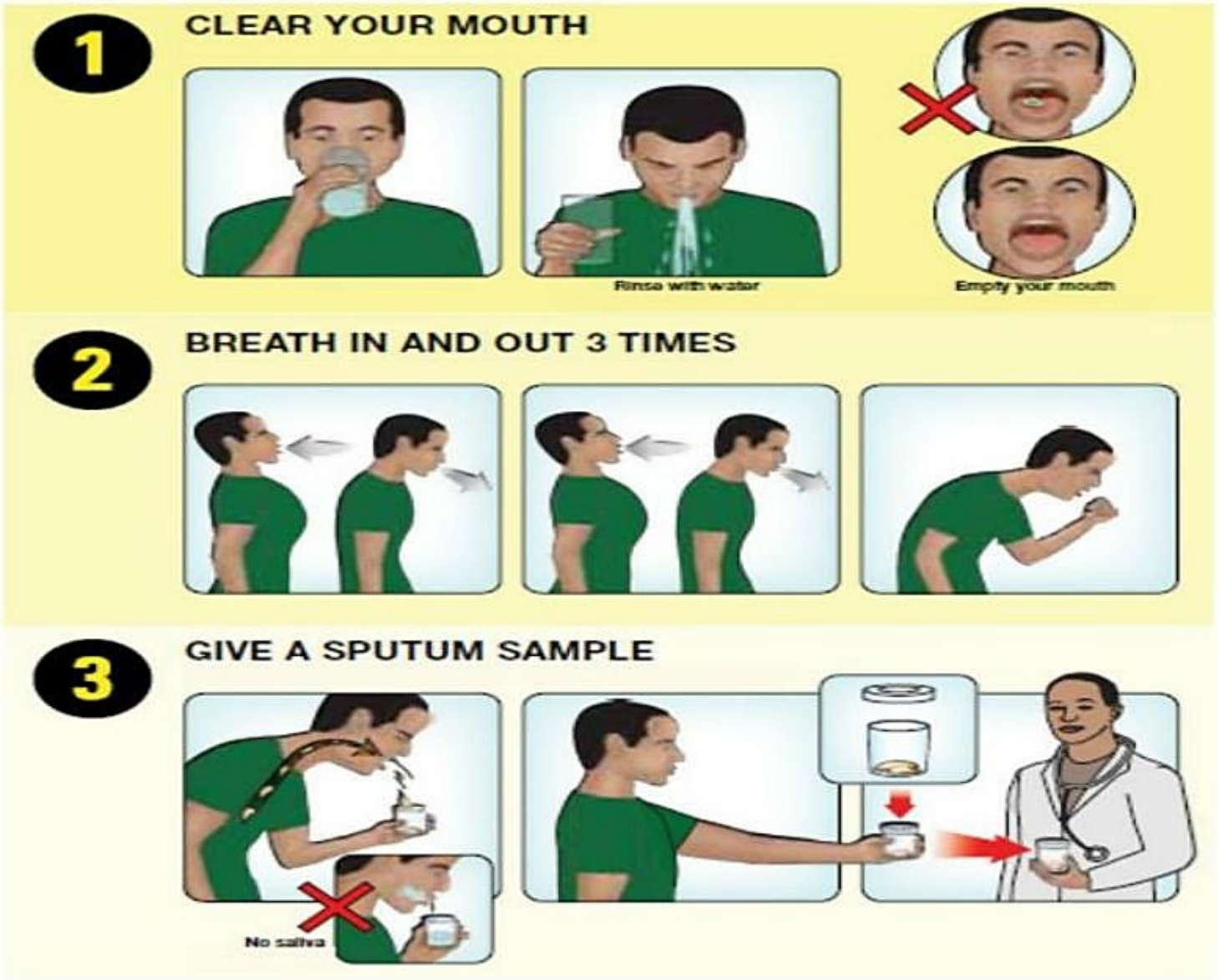
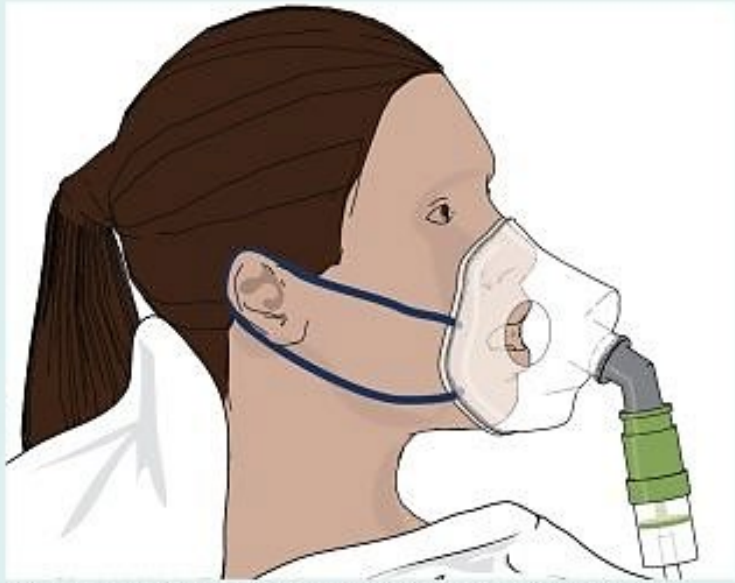
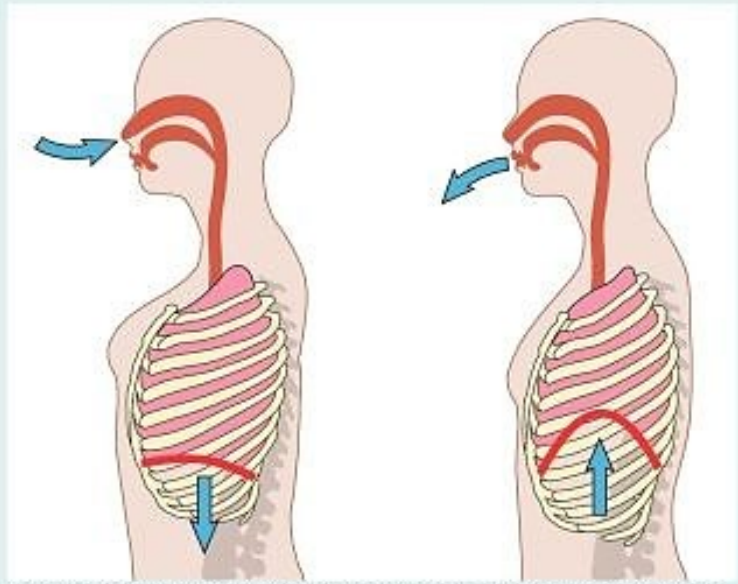


Fig 2. **The procedure**

2a. Position the patient upright in a chair or in bed in the Fowler position



2b. A sodium chloride nebuliser can help loosen secretions

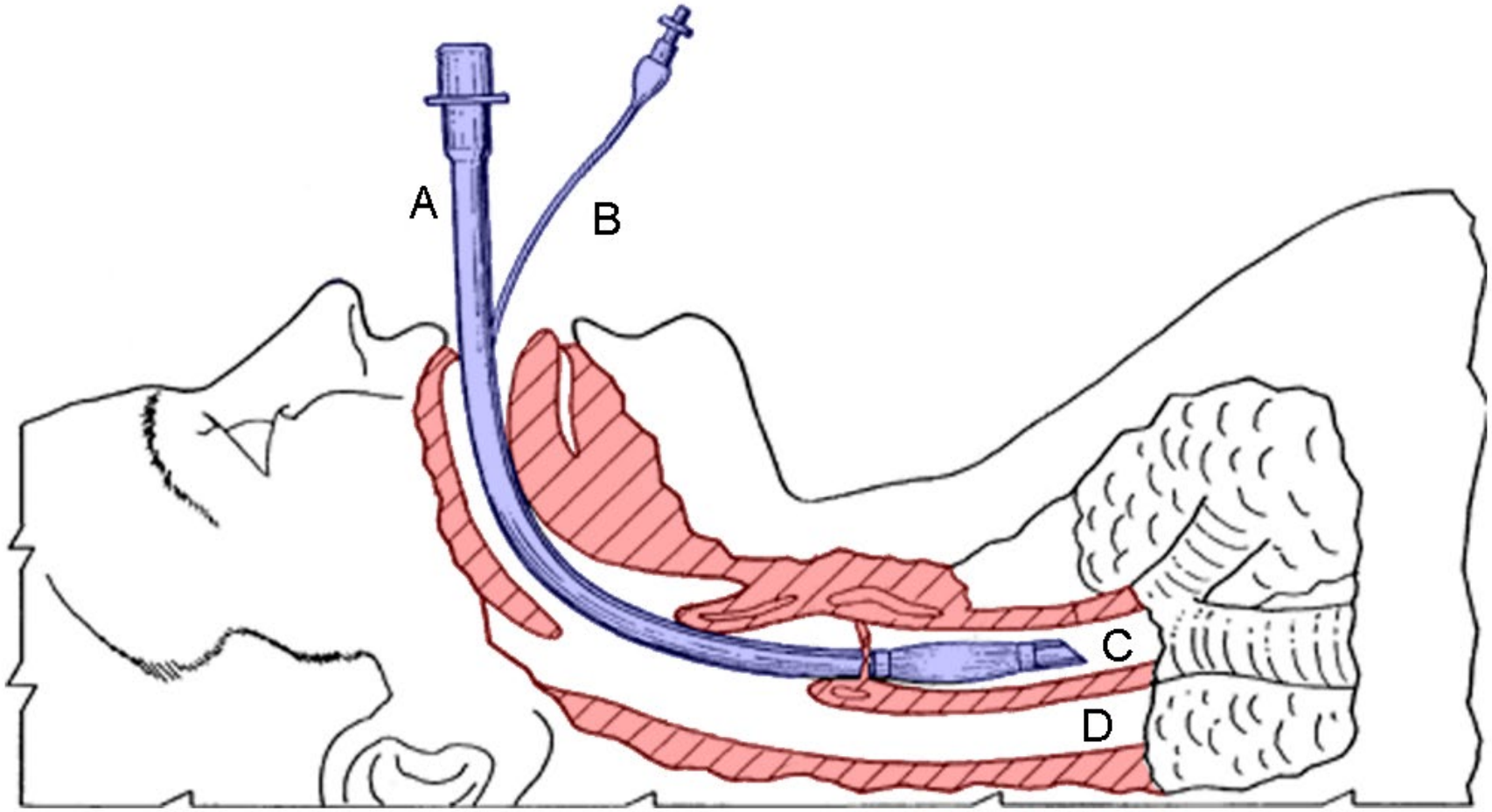


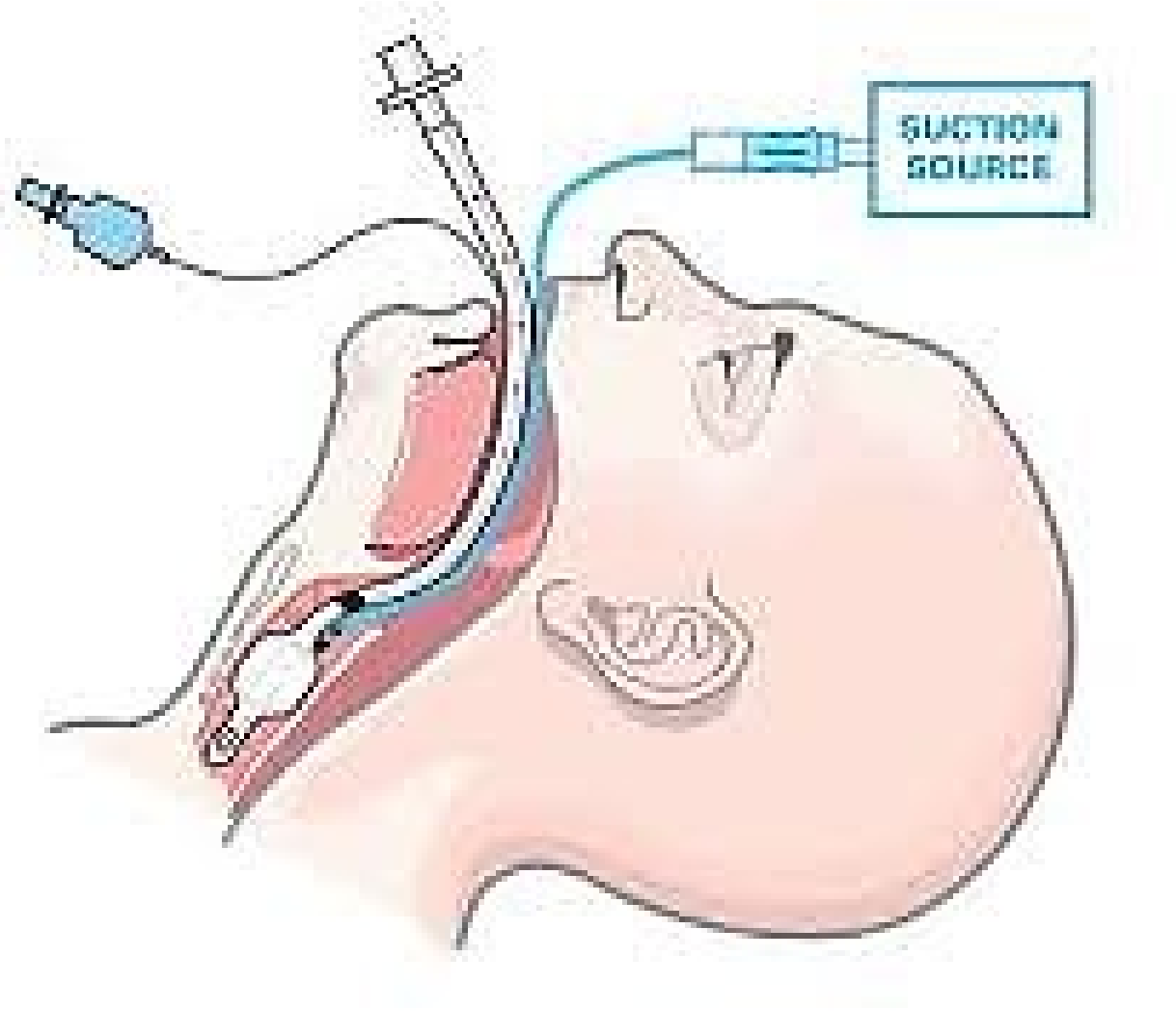
2c. Ask the patient to take deep breaths – in through the nose and out through the mouth – to help loosen secretions

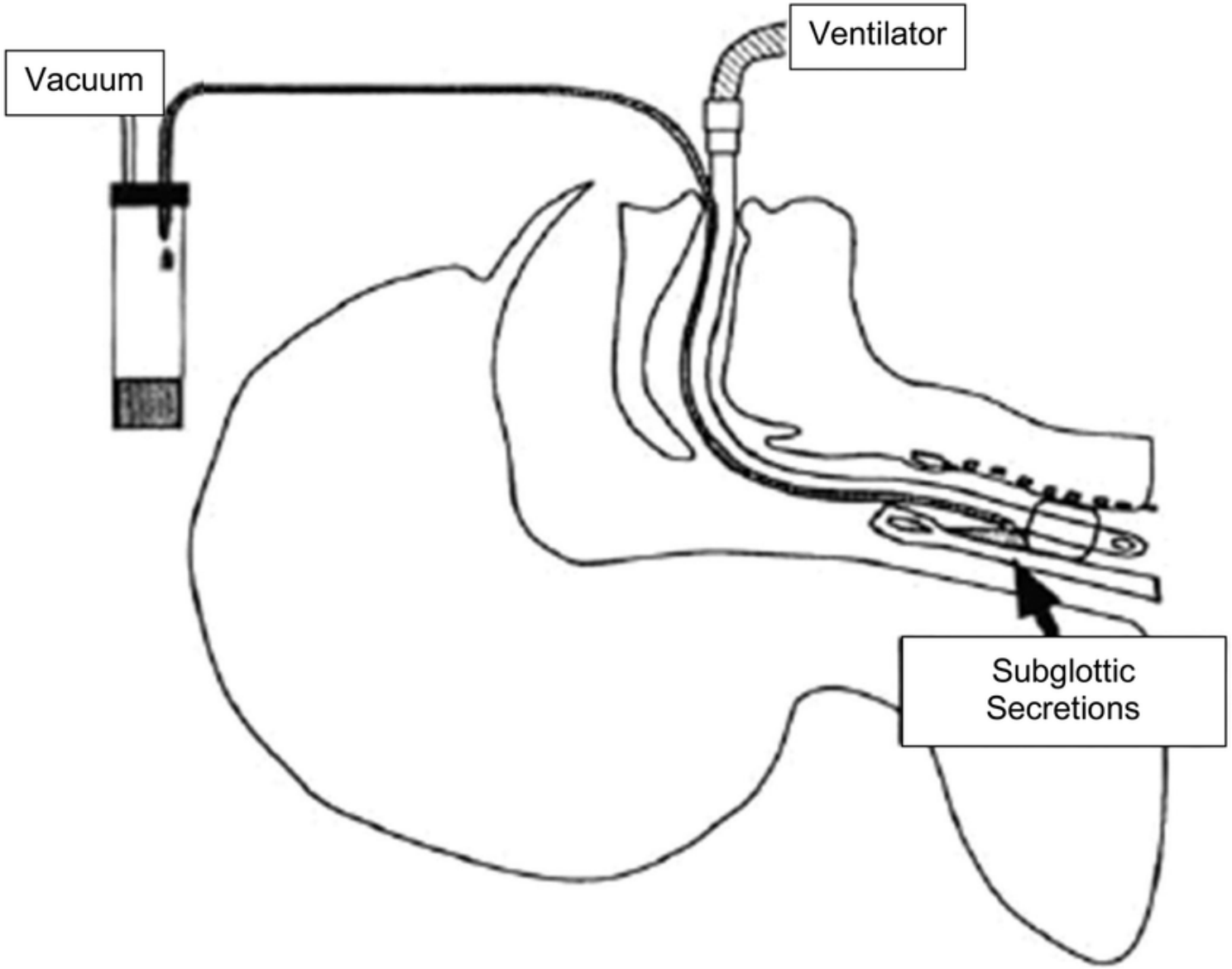
2d. Collect the specimen in the pot and seal it with a lid to prevent contamination and reduce the risk of cross infection

Hospital Acquired Pneumonia Diagnosis

- American College of Chest Physicians: Clinical findings are not sufficient for definitive diagnosis
- Qualitative culture or endotracheal sputum has poor predictive value
- Bronchoscopy is recommended by many pulmonologists
  - Bronchial brushings
  - Bronchial washes
  - Protected specimen brushing
  - Bronchoalveolar lavage specimens (BAL)
  - Transbronchial biopsy

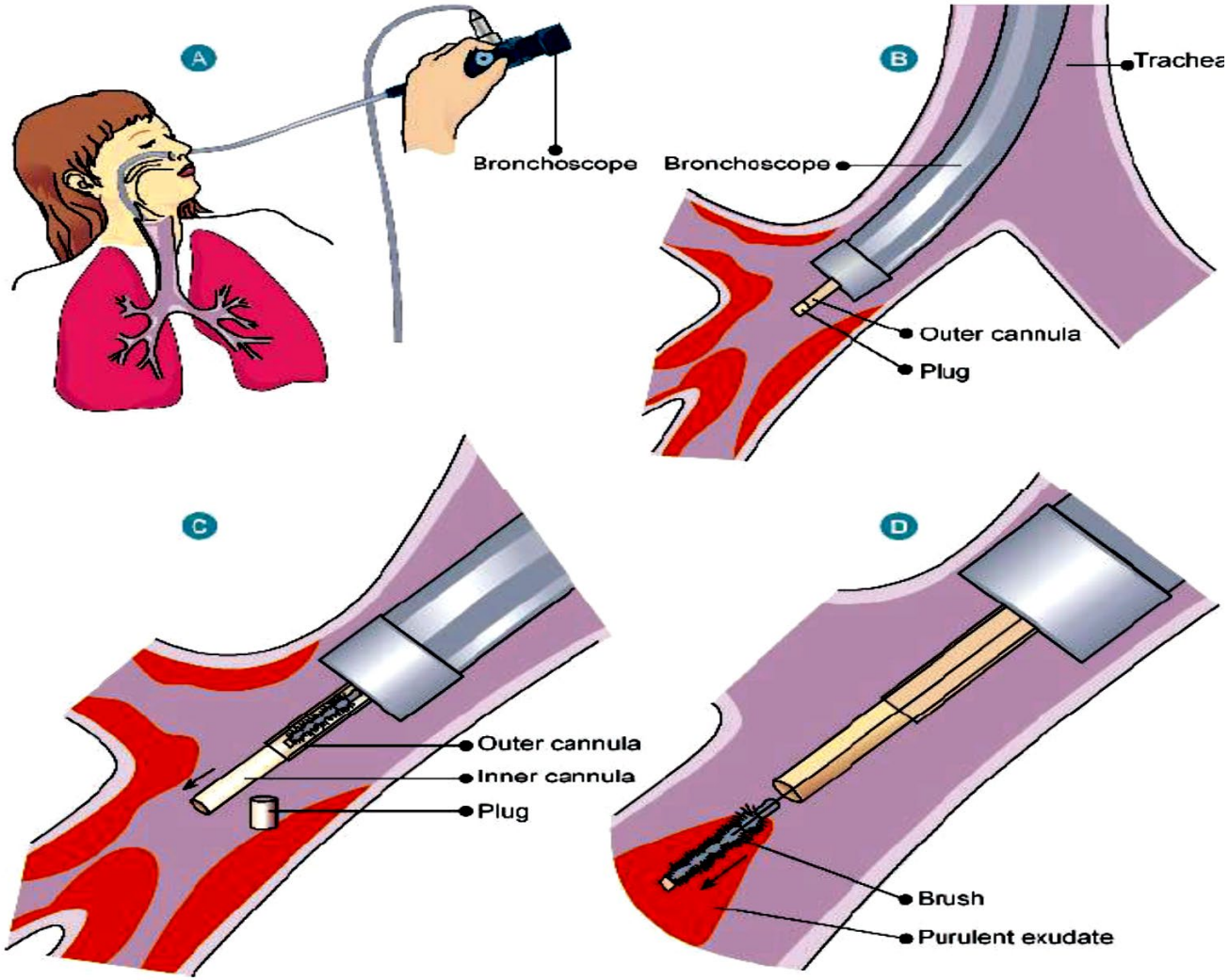

A
B
C
D


SUCTION
SOURCE


Ventilator
Vacuum
Subglottic
Secretions

Processing Bronchoscopy Specimens

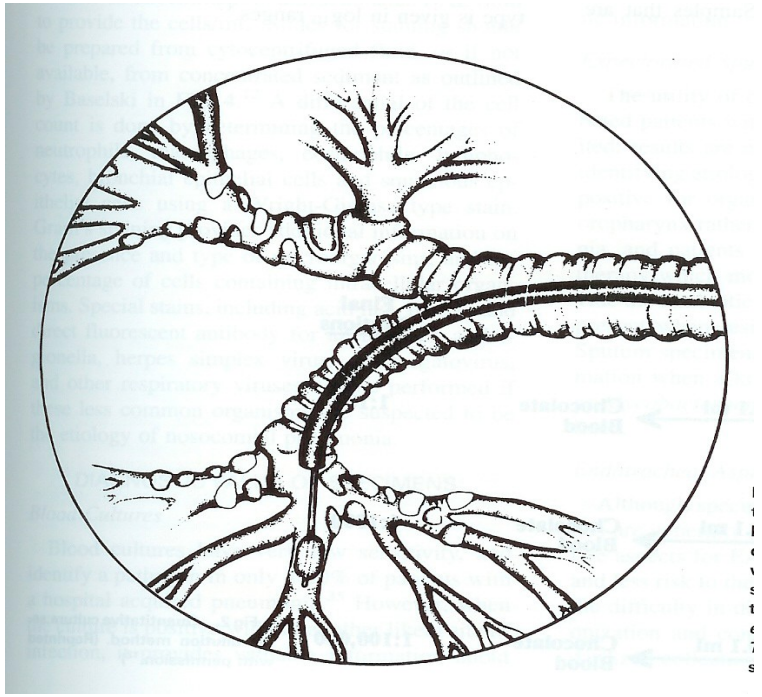
- Bronchoscopy brush protected
  - Aerobic bacterial culture and Gram stain
  - Anaerobic bacterial culture
  - Limited volume
- Bronchoscopy brush, unprotected
  - No anaerobic culture
  - Limited volume
- Bronchial washings
  - Useful only for pneumonia caused by strict pathogens
  - Reasonable requests: Mtb, Fungi, *Legionella*, *Pneumocystis*
- Bronchoalveolar lavage
  - No anaerobe culture
  - Amenable to extensive testing for all opportunistic pathogens



شکل ۳-۲: مراحل جمع‌آوری ترشحات برنش توسط کاتتر با حفاظ

Respiratory Specimens

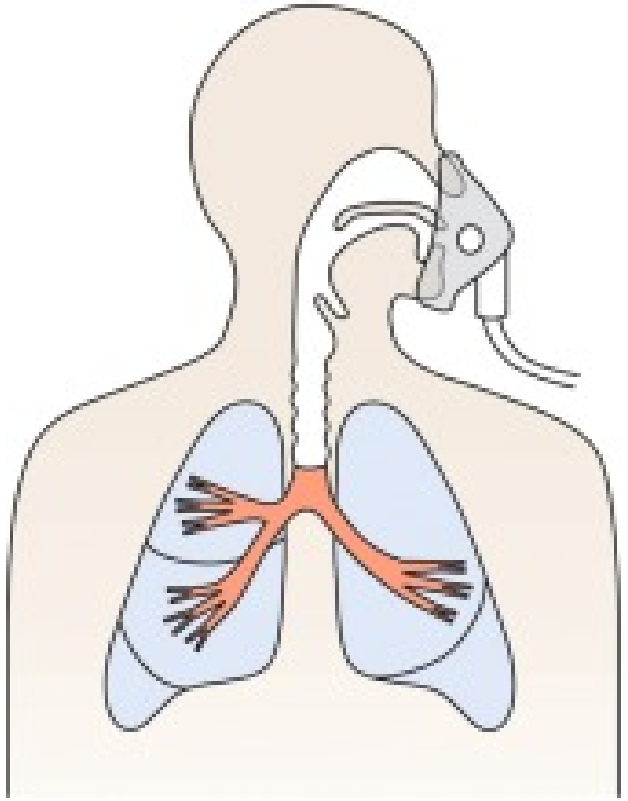
- Protected Brush Specimen
  - To procure uncontaminated lower airway secretions
  - Brush within2 catheters



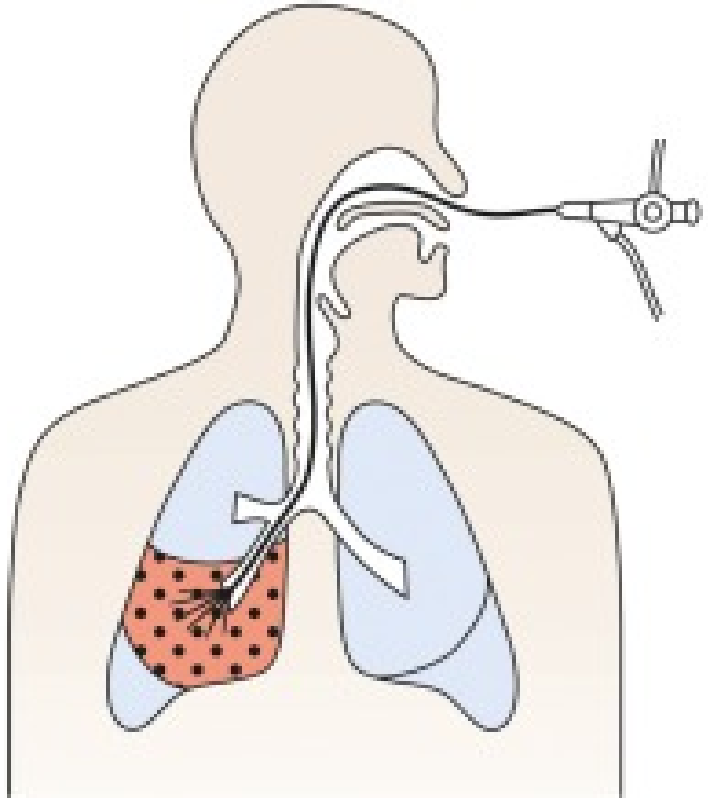
Respiratory Specimens

- Bronchoalveolar Lavage (BAL)
  - Samples large area of the lung
  - Performed using a bronchoscope
  - 100 to 250 ml of saline injected
  - Injected saline along with secretions is collected by aspiration
- Transthoracic Aspiration
  - Involves percutaneous introduction of a needle directly into the infiltrate

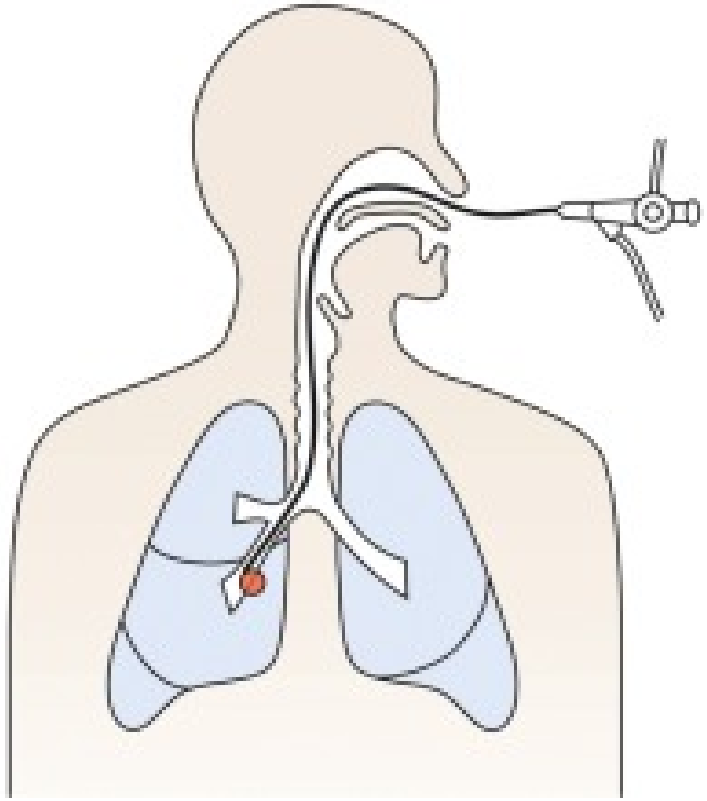
Induced sputum

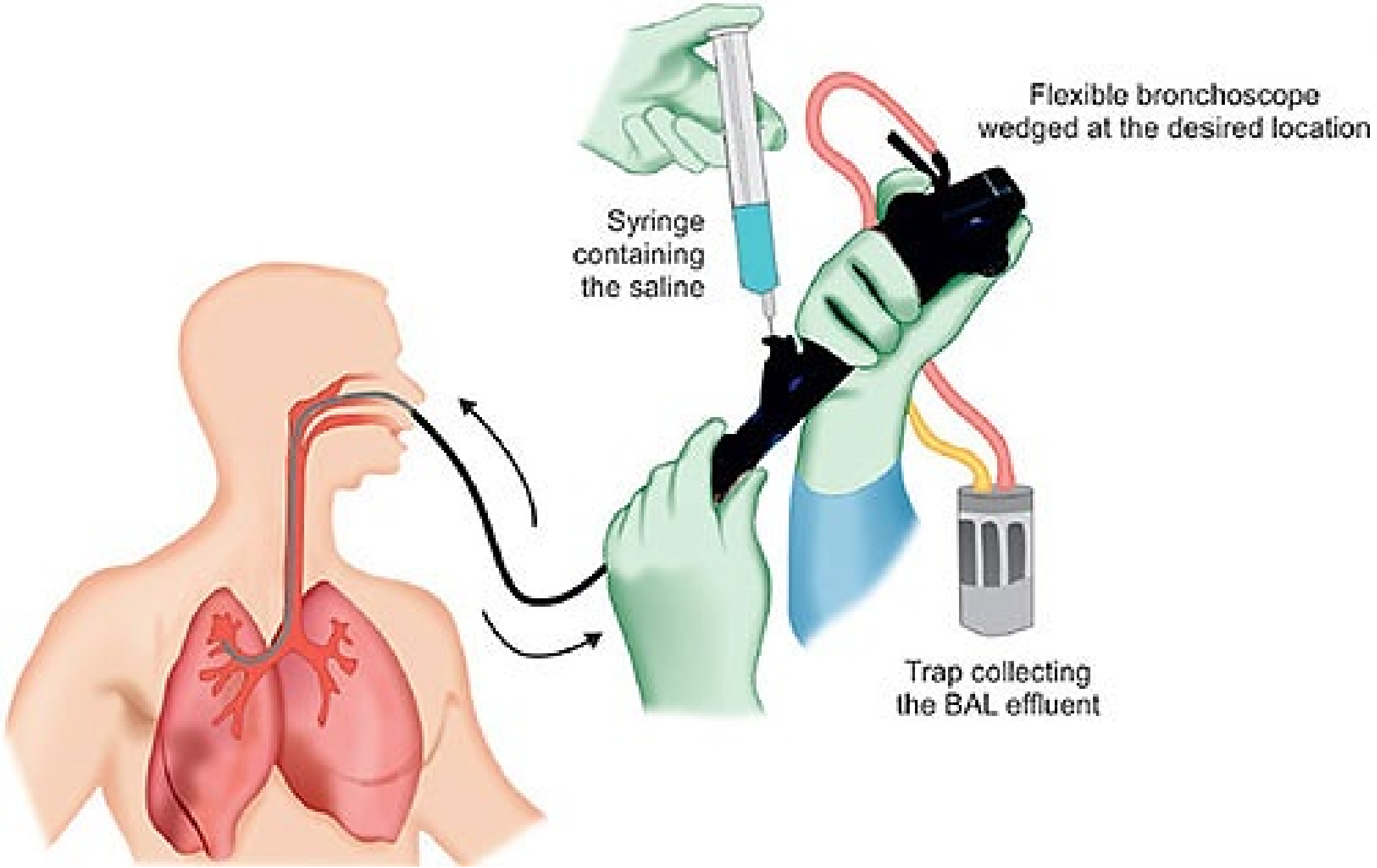


Bronchoalveolar lavage



Endobronchial biopsy




Flexible bronchoscope
wedged at the desired location
Syringe
containing
the saline
Trap collecting
the BAL effluent

Bronchoalveolar Lavage (BAL) Specimen Acceptability

- Microscopic examination of Gram-stained smear
  - Acceptable
    - <1% of cells present are squamous epithelial cells
  - Unacceptable
    - >1% of cells present are squamous epithelial cells

Thorpe JE et. al. 1987. Bronchoalveolar lavage for diagnosing acute bacterial pneumonia. *J. Infect. Dis.* 155:855-861