



Amal

Lab. 2

Alghamdi

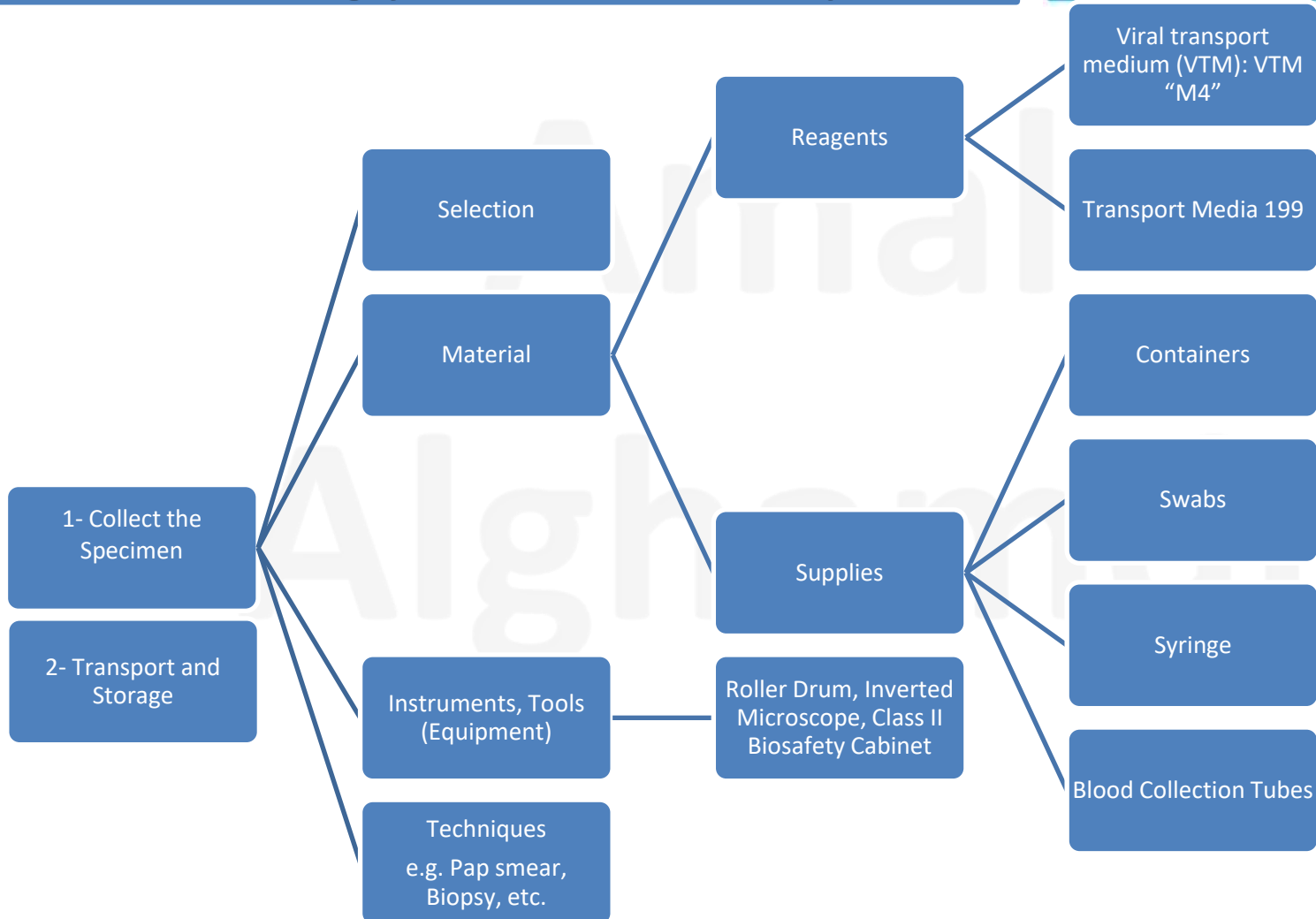
Practical 450 MBIO

Learning outcomes



- The techniques used to collect the samples from the target organs.
- The material we should use in medical virology lab.
- The proper transport and storage viral specimens.

Setting Up A Clinical Virology Laboratory





1-Collection of Virus specimens

A-Selection of specimens

B-Material used for sample collection along with tools and instruments used.

C-Techniques to collect samples

2- Transport and Storage of Specimens

- **Transport** the specimens as directed so as to maintain viability and minimize overgrowth with contaminating organisms.
- **Place** each specimen into a separate container labeled with the patient's name and identification number, the collection site, the date of collection, and the time of the collection.



A-Selection of specimens

- To ensure accurate diagnosis of viral disease, it is important to select the appropriate specimens.
- The specimen should be collected from the target organ most closely associated with clinical symptoms to identify the etiologic agent responsible for the patient's disease.
- It should be collected during the acute phase of infection when viral concentration is at its maximum. Autopsy samples need to be collected as soon as possible after death before tissues start decomposing.

B) Materials:

1- Reagents: Viral transport medium (VTM):

A- Commercially Available:

Contains Salt solution with antimicrobial agents to prevent specimen drying, maintains viral viability and retards the growth of microbial contaminants

- The VTM 'M4'. Tubes containing 2-3 mL VTM are used for swab specimens,



B- Manually Prepared:

- Transport Media 199
- PBS-Glycerol transport medium

B) Materials:

• 2- Supplies:

A- Containers:

Sterile, leak-proof, screw-cap containers including urine cups, disposable centrifuge tubes (15 and 50 mL), suitable for holding 1-2 mL of VTM.

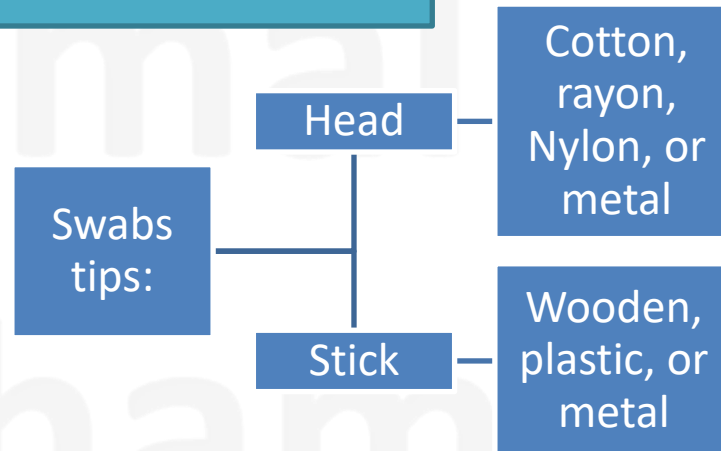


B) Materials:

• 2- Supplies:

B- Swabs:

Sterile cotton, or rayon-tipped swabs with plastic or aluminum shafts small-tip flexible swabs are used for certain samples such as urethral swabs.

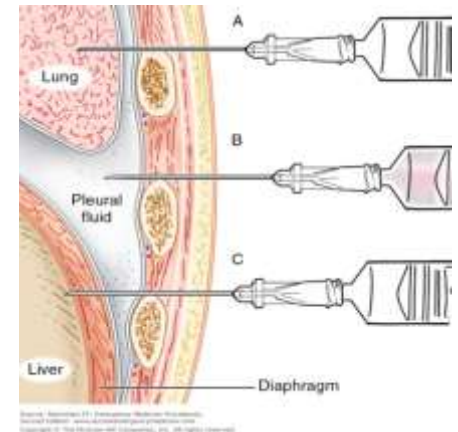


B) Materials:

- 2- Supplies:

C- Syringes:

Tuberculin syringe with 26- or 27-gauge needle for aspirating vesicular fluid.



B) Materials:

• 2- Supplies:

D – Blood Collecting:

- Blood collection tubes containing anticoagulant (ACD) for plasma.
 - 5-10 ml of anti-coagulated blood for CMV (associated with peripheral blood leukocytes) viremia.
 - Heparinized, citrated, or ethylenediaminetetraacetic acid (EDTA) is acceptable for CMV detection.



3- instruments, Tools (Equipment):

Roller drum



used to hold cell culture tubes during incubation. Slow rotation continually bathes the cells in the medium.

Inverted microscope



used to examine cell monolayers growing attached to the inside surface beneath the liquid medium.

Class II Biosafety Cabinet



Used in a clinical virology laboratory.

Surgical Tools

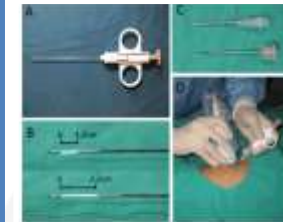
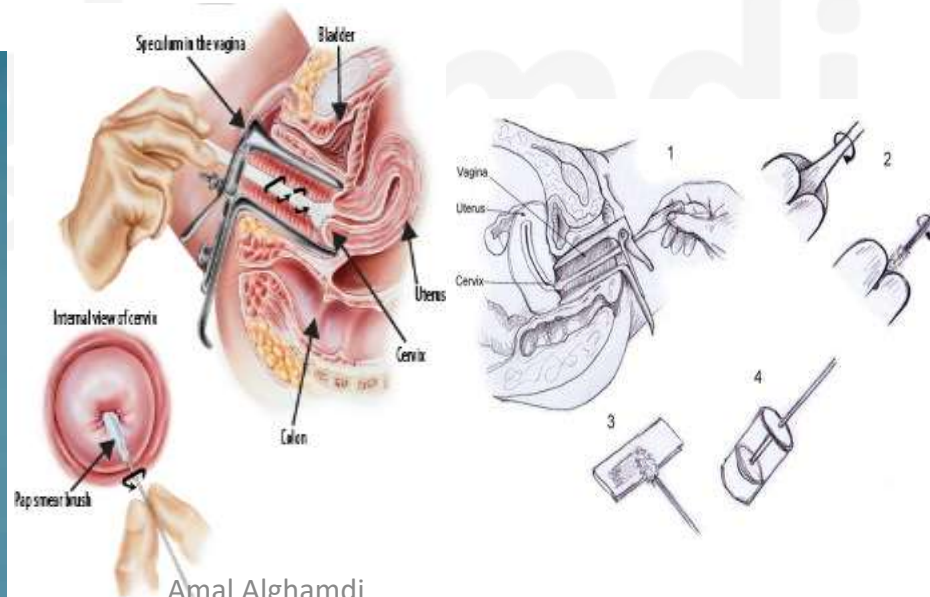


Figure 1: SuperCore Biopsy Instrument.

Super Core biopsy instrument.

An Example: Pap smear

A Pap smear (Papanicolau smear; also known as the Pap test) is a screening test for cervical cancer. The test itself involves collection of a sample of cells from a woman's cervix (the end of the uterus that extends into the vagina) during a routine pelvic exam. The cells are placed on a glass slide and stained with a substance known as Papanicolau stain. The stained cells are then examined under a microscope to look for pre-malignant (before-cancer) or malignant (cancer) changes.





4-How to collect samples (The techniques)

1-Swabs:

Rectal swab: Insert swab 4-6 cm and roll against mucosa. Place swab in 1-2 ml of sterile saline or viral transport media, break off the swab into medium.

Vesicle or lesion swab: Open lesion carefully using a sterile instrument. Moisten a sterile swab with sterile saline or other transport media and collect cells from open lesion. Place swab in 1-2 ml of sterile saline or viral transport media.

Ocular swab: Collect from lower conjunctiva using a swab moistened with sterile saline. Place swab in 1-2 ml of sterile saline or viral transport media.



Throat swab (THRT): Swab posterior throat and tonsil area and place swab in 1-2 ml of sterile saline or viral transport media.



- **Nasal swab:** Swab nostrils separately and place swabs in 1-2 ml of sterile saline or viral transport media.
- **Nasopharyngeal swab (NP):** Insert sterile swab through nostril into nasopharynx and rotate several times. Remove and place swab in 1-2 ml of sterile saline or viral transport media.

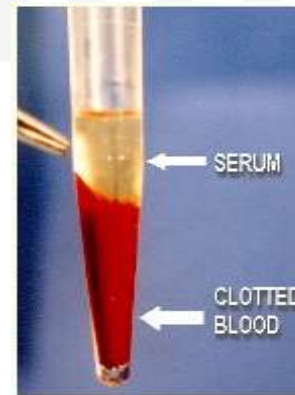
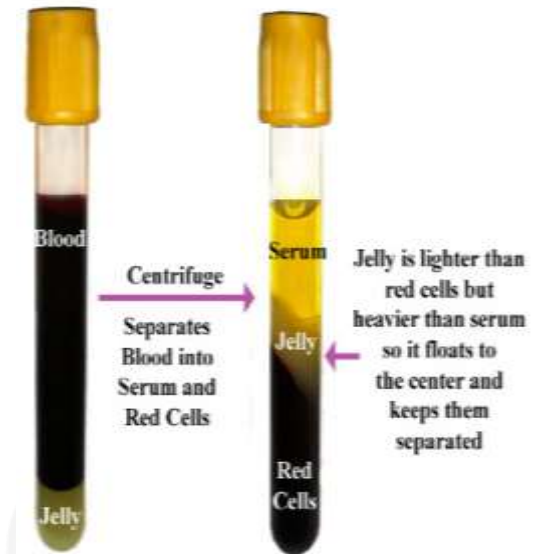


2-Blood (BLD):

1- Whole blood: Collect in EDTA (purple top) tube.

2- Serum: Collect in red top tube. Centrifuge and remove from clot if possible.

*Note: When requesting antibody titers, send paired samples for the most accurate results.



3- Aspirate:

1- Nasal Aspirate: Insert suction device through nostrils into nasopharynx. Aspirate fluid while removing suction device. Flush device with sterile saline and collect in a sealed container.



2-Throat Aspirate

3- Fecal Aspirate

4- Tissue or Biopsy (BX):

Place in sterile container or VTM with a small amount of sterile saline to keep moist.





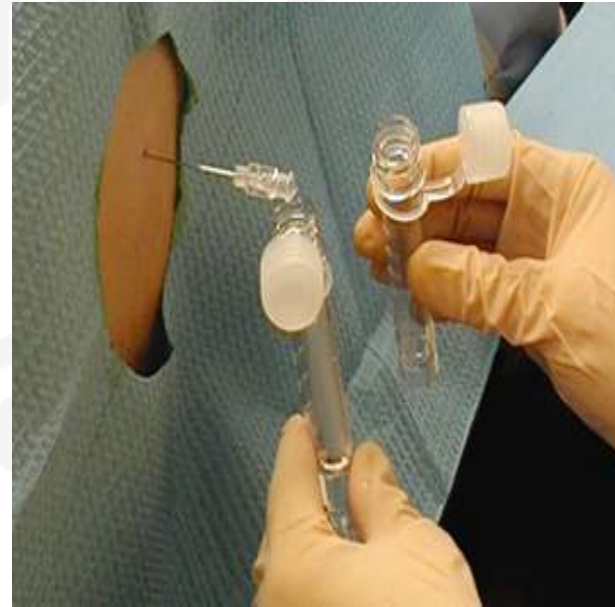
5- Self-collected:

- 1-Semen:** Collect in semen straw and transfer immediately into liquid nitrogen.
- 2- Urine (URN):** Collect at least 1 hour after last urination. Transport specimen in polypropylene containers, which are provided on request.
- 3- Feces:** Place 2-4 grams inside a sterile sealed container.

6- CSF: Collect the cerebrospinal fluid in sterile container.



Regular needle
CSF needle





Laboratory processing of viral specimen

TABLE 65-4 Laboratory Processing of Viral Specimens

Source	Specimen	Processing*	Cells for Detection of Common Viruses
Blood	Anticoagulated blood	Separate leukocytes (see Procedure 65-1)	PMK, HDF, HEp-2
Cerebrospinal fluid (CSF)	1 mL CSF	Inoculate directly	PMK, HDF, HEp-2
Stool or rectal swab	Pea-sized aliquot of feces	Place in 2 mL of viral transport medium vortex. Centrifuge at 1000× g for 15 min and use supernatant fluid for inoculum	PMK, HDF, HEp-2
Genital, skin	Vesicle fluid or scraping	Emulsify in viral transport medium	HDF
Miscellaneous	Swab, fluids	Emulsify in viral transport medium Fluid, inoculate directly	PMK, HDF, HEp-2
Respiratory tract	Nasopharyngeal secretions, throat swab, respiratory tract washings, sputum	Dilute with viral transport medium	PMK, HDF, HEp-2
Tissue	Tissue in sterile container	Mince with sterile scalpel and scissors and gently grind. Prepare 20% suspension in viral transport medium. Centrifuge at 1000× g for 15 min and use supernatant fluid for inoculum.	PMK, HDF, HEp-2
Urine	Midstream specimen	Clear: Inoculate directly. Turbid: Centrifuge at 1000× g for 15 min and use supernatant fluid for inocula.	HDF, HEp-2 (if adenovirus suspected)

HDF, Human diploid fibroblast; HEp-2, human epidermoid; PMK, primary monkey kidney.

*All inocula into tissue culture tubes are 0.25 mL volumes.



Laboratory processing of viral specimen

Specimen	Optimal specimen collection schedule	Volume	Specimen handling instructions
Cerebrospinal fluid (CSF)	within 7 days of onset	1-2 mL	
Amniotic fluid		3 mL	
Vitreous fluid (eye)		1 mL	
Urine	Within 2 weeks of onset	5 mL	
Blood		5-7 mL	In yellow ACD tube
Bone marrow aspirate		2-5 mL	In yellow ACD tube
Bronchoalveolar lavage (BAL)		3-5 mL	
Swabs (nasal, throat, nasopharyngeal, vesicle/lesion, rectal, eye/conjunctive, genital)	NP: within 5 days VESCL/GEN swabs - as early as possible	swab in M4	In viral transplant media M4
Aspirates (nasal, tracheal, sinus, vesicle)		1-2 mL	
Nasal wash	Within 5 days of onset	3-5 mL	
Feces	Within 2 weeks of onset	5 mL	In container without preservatives
Tissue		variable	In sterile saline or viral transport media



Specimen for viral diseases' diagnosis

Disease Categories and Probable Viral Agent	Season of Most Common Occurrence	Throat/ Nasopharynx	Stool	CSF	Urine	Other
Respiratory						
Adenoviruses	Y	++++				
Influenza virus	W	++++				
Parainfluenza virus	Y	++++				
Respiratory syncytial virus (RSV)	W	++++				
Metapneumovirus	W	++++				
Rhinoviruses	Y					Nasal (+++)
SARS coronavirus	W	++++				
Sin nombre virus	SP, S					Serum for antibody detection
Dermatologic and Mucous Membrane						
VESICULAR						
Enterovirus	S, F	++	+++			Vesicle fluid or scraping
Herpes simplex virus [†]	Y					Vesicle fluid or scraping
Varicella-zoster virus [†]	Y	++				Vesicle fluid or scraping
Monkeypox	Y					Vesicle fluid or scraping
EXANTHEMATOUS						
Enterovirus	S, F	+++	++			
Measles	Y	++			++	Serum for antibody detection
Rubella	Y				++	Serum for antibody detection
Parvovirus	Y					Serum for antibody detection amniotic fluid (PCR)



Serology Tests for Hepatitis Virus

Disease	Virus	Diagnostic Tests
Hepatitis A	Enterovirus 72	Antibody to Hepatitis A virus (IgG and IgM)
Hepatitis B	Hepadnavirus	Hepatitis B surface-antigen (HBsAg) Hepatitis B early-antigen (HBeAg) Anti-HBsAg Anti-HBeAg Anti-HB core antigen
Hepatitis C	Flavivirus	Antibody to hepatitis C virus
Hepatitis D	Delta agent (hepatitis D virus)	Antibody to delta agent
Hepatitis E	Calicivirus-like (herpes virus)	Antibody to hepatitis E virus



2- Transport and Storage of Specimens

- Use provided transport bag usually combined with a request form.
- Samples should reach the laboratory within 24 hrs. If this is not possible refrigerate
- Store samples in a short term transport storage 4°C degrees Celsius after that in a long term transport(>72hours) storage-70°C with liquid nitrogen
- Swab samples can be kept a 2 - 27 degrees Celsius for up to 4 - 6 days and NEVER send dry swabs.



A transport bag



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9006 501 016 8

GFQ90W DFU-D-4380

Please fill out patient data clearly in block letters.

Family name: _____
 Forename: _____
 M. No.: _____
 Physician: _____

Specimen data
 Sampling date: _____ Time: _____

Sample type

<input type="checkbox"/> Serum (S)	<input type="checkbox"/> EDTA-p (ED)	<input type="checkbox"/> CSF	<input type="checkbox"/> Serum (S)
<input type="checkbox"/> EDTA-p (ED)	<input type="checkbox"/> heparin (H)	<input type="checkbox"/> Cytoceres (C)	<input type="checkbox"/> Swab (SW)
<input type="checkbox"/> Heparin (H)	<input type="checkbox"/> Citrat (C)	<input type="checkbox"/> Woodcock (W)	<input type="checkbox"/> Tissue (T)
<input type="checkbox"/> Urine (U)	<input type="checkbox"/> AFKSB-Puff (P)	<input type="checkbox"/> Urine (U)	

Other data
 Patient's age: _____ sex: male female
 Patient's height: _____
 Patient's weight: _____
 Patient's height: _____
 No. of tubes set: _____

Analyses required (Please note sample requirements in the Test List)

<input type="checkbox"/> E-TF (thrombinogen)	<input type="checkbox"/> E-Chlamydia trach. IgA	<input type="checkbox"/> E-HIV genotyping	<input type="checkbox"/> E-Fsp enter
<input type="checkbox"/> E-AIG	<input type="checkbox"/> E-Cocci	<input type="checkbox"/> E-HbA1c	<input type="checkbox"/> E-Phycocyanin
<input type="checkbox"/> E-Anti-mycoplasma	<input type="checkbox"/> E-DRV-ant.	<input type="checkbox"/> E-Rib-oncogen	<input type="checkbox"/> E-MSL screen
<input type="checkbox"/> E-ACE2-antib.	<input type="checkbox"/> E-Carotid	<input type="checkbox"/> E-HCV-ant.	<input type="checkbox"/> E-Prevalence
<input type="checkbox"/> E-AGP - Capacity	<input type="checkbox"/> E-Carotid	<input type="checkbox"/> E-HIV genotyping	<input type="checkbox"/> E-Prostate
<input type="checkbox"/> E-Alcohol	<input type="checkbox"/> E-Creatinine	<input type="checkbox"/> E-HIV RNA-ant.	<input type="checkbox"/> E-Protein-Carotid
<input type="checkbox"/> E-Albumin	<input type="checkbox"/> E-Creatinin	<input type="checkbox"/> E-HIV RNA-ant.	<input type="checkbox"/> E-Protein-Carotid
<input type="checkbox"/> E-Aluminium	<input type="checkbox"/> E-CRP	<input type="checkbox"/> E-Histocyt. papill. ant.	<input type="checkbox"/> E-PSA structure
<input type="checkbox"/> E-Anti-act. part.	<input type="checkbox"/> E-CSP-ribonuclein	<input type="checkbox"/> E-Histology screen	<input type="checkbox"/> E-PTA-ant.
<input type="checkbox"/> E-Anti-act. part.	<input type="checkbox"/> E-CHGA-Ig	<input type="checkbox"/> E-Hesperophila	<input type="checkbox"/> E-Sant
<input type="checkbox"/> E-Asa	<input type="checkbox"/> E-DNA-ant. (IgG/IgM)	<input type="checkbox"/> E-Hesperophila	<input type="checkbox"/> E-Sant
<input type="checkbox"/> E-ASA-C	<input type="checkbox"/> E-Drug screen	<input type="checkbox"/> E-HGH	<input type="checkbox"/> E-Tandem
<input type="checkbox"/> E-ASA-F	<input type="checkbox"/> E-Drug-screening system	<input type="checkbox"/> E-Histology	<input type="checkbox"/> E-Tetocyan
<input type="checkbox"/> E-Azotemia	<input type="checkbox"/> E-DRV-ant.	<input type="checkbox"/> E-HV-13-ant.	<input type="checkbox"/> E-Tetocyan
<input type="checkbox"/> E-Azotemia	<input type="checkbox"/> E-SMA-ant.	<input type="checkbox"/> E-HV-1 RNA-ant.	<input type="checkbox"/> E-Thrombocyt. ant.
<input type="checkbox"/> E-B-2-Microglobulin	<input type="checkbox"/> E-Endothelin (IgG-ant.)	<input type="checkbox"/> E-HLA-B27	<input type="checkbox"/> E-Thyroid-ant. (SAM-TAG)
<input type="checkbox"/> E-Bla-act. test	<input type="checkbox"/> E-Enzymogram (IgG-ant.)	<input type="checkbox"/> E-HLA Typing (A, B, C)	<input type="checkbox"/> E-TOR2
<input type="checkbox"/> E-C-Comp.	<input type="checkbox"/> E-Factor VIII-ant.	<input type="checkbox"/> E-HLA Typing (A, C, DR, DM)	<input type="checkbox"/> E-Tandem
<input type="checkbox"/> E-C-Comp.	<input type="checkbox"/> E-Factor VIII-ant. eq.	<input type="checkbox"/> E-Hemostatic	<input type="checkbox"/> E-Thrombin-ant.
<input type="checkbox"/> E-CA150	<input type="checkbox"/> E-Factor VIII-ant. do. fac.	<input type="checkbox"/> E-IL-18-ant.	<input type="checkbox"/> E-TSH
<input type="checkbox"/> E-CA150	<input type="checkbox"/> E-Ferritin	<input type="checkbox"/> E-IgG	<input type="checkbox"/> E-TSH receptor-ant.
<input type="checkbox"/> E-CA19.9	<input type="checkbox"/> E-Fibrin	<input type="checkbox"/> E-IgG-I	<input type="checkbox"/> E-TSH screen
<input type="checkbox"/> E-CA19.9	<input type="checkbox"/> E-Fibrin	<input type="checkbox"/> E-IgG-II	<input type="checkbox"/> E-Vitamin B12
<input type="checkbox"/> E-C-Apple	<input type="checkbox"/> E-Fibrin-ant.	<input type="checkbox"/> E-Immunochem.	<input type="checkbox"/> E-Vitamin D (1,25)
<input type="checkbox"/> E-Carotid-ant.	<input type="checkbox"/> E-Free Testosterone	<input type="checkbox"/> E-Insulin	<input type="checkbox"/> E-Vitamin D (25)
<input type="checkbox"/> E-Carotid-ant.	<input type="checkbox"/> E-FSH	<input type="checkbox"/> E-Insulin	<input type="checkbox"/> E-Vitamin D (25)
<input type="checkbox"/> E-Carotid-ant.	<input type="checkbox"/> E-FTS	<input type="checkbox"/> E-IP-1	<input type="checkbox"/> E-VISA
<input type="checkbox"/> E-Calcitonin	<input type="checkbox"/> E-FTN	<input type="checkbox"/> E-IgA-antibod. / infodiv	Other Analyses
<input type="checkbox"/> E-Calcitonin	<input type="checkbox"/> E-S-IgD	<input type="checkbox"/> E-IgM-ant.	<input type="checkbox"/>
<input type="checkbox"/> E-CAM (Herceptin)	<input type="checkbox"/> E-Squamous screen	<input type="checkbox"/> E-Immunochem. (PSA)	<input type="checkbox"/>
<input type="checkbox"/> E-COP	<input type="checkbox"/> E-Spleen	<input type="checkbox"/> E-Immunohist. (PSA)	<input type="checkbox"/>
<input type="checkbox"/> E-COP-ant.	<input type="checkbox"/> E-Statist. ant.	<input type="checkbox"/> E-Immunohist. ant.	<input type="checkbox"/>
<input type="checkbox"/> E-Corticosteron	<input type="checkbox"/> E-Thrombocyt. ant.	<input type="checkbox"/> E-Myeloperox. ant.	<input type="checkbox"/>
<input type="checkbox"/> E-Chlamydia pneum. ant.	<input type="checkbox"/> E-Thrombocyt. ant.	<input type="checkbox"/> E-Nicotin. ant.	<input type="checkbox"/>
<input type="checkbox"/> E-Chlamydia trach. ant.	<input type="checkbox"/> E-HIV-1/2-ant.	<input type="checkbox"/> E-Thrombocyt. ant.	<input type="checkbox"/>
<input type="checkbox"/> E-Chlamydia trach. serop.	<input type="checkbox"/> E-HIV-1/2-ant.	<input type="checkbox"/> E-Thrombocyt. ant.	<input type="checkbox"/>

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2017-18

The request form



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- <http://www.copanusa.com/education/videos/>

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