

# 450MIC

lab2

# Learning outcome

- Study how to collect right samples from the target organs
- Learn which material we should use in medical virology lab
- Learn how to transport and storage viral specimens

# 1-Collection of Virus specimens

**A-Selection of specimens**

**B-Material for sample collection**

**C-How to collect samples**

# 2- Transport and Storage of Specimens

# 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.
- 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.

# B) Materials for Samples collection

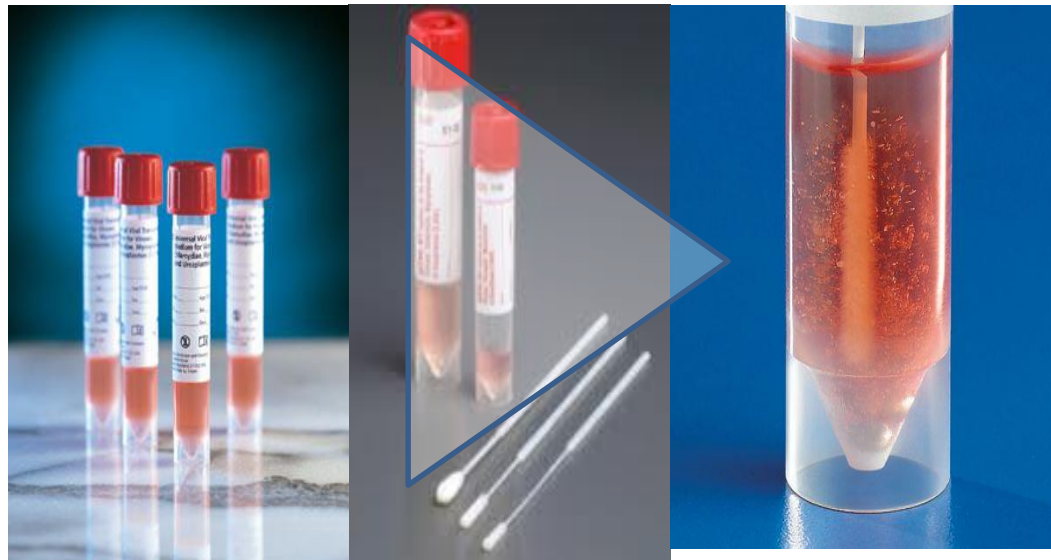
## 1- Reagents

– Viral transport medium (VTM):

### 1-Commercial

contains Salt solution with antimicrobial agents to prevents 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
- while those with 5-7 mL VTM are suitable for tissue samples.



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2017

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# 2-Prepared

## A. Transport medium 199

1. Tissue culture medium 199 containing 0.5% bovine serum albumin (BSA)
2. To 1 litre of above add:
  - benzylpenicillin ( $2 \times 10^6$  IU/litre)
  - streptomycin (200 mg/litre)
  - polymyxin B ( $2 \times 10^6$  IU/litre)
  - gentamicin (250 mg/litre)
  - nystatin ( $0.5 \times 10^6$  IU/litre)
  - ofloxacin hydrochloride (60 mg/litre), and sulfamethoxazole (0.2 g/litre)
3. Sterilize by filtration and distribute in 1.0–2.0 ml volumes in screw-capped tubes.

# B. PBS-Glycerol transport medium

## 1. Phosphate-buffered saline (PBS):

- NaCl 8g
- KCl 0.2g
- Na<sub>2</sub>HPO<sub>4</sub> 1.44g
- KH<sub>2</sub>PO<sub>4</sub> 0.24g
- Distilled water to make 1 litre

## 2. Autoclave PBS and mix 1:1 with sterile glycerol to make 1 litre

## 3. To 1 litre PBS/glycerol add:

- benzylpenicillin (2 x 10<sup>6</sup> IU/litre)
- streptomycin (200 mg/litre) – polymyxin B (2 x 10<sup>6</sup> IU/litre)
- gentamicin (250 mg/litre)
- nystatin (0.5 x 10<sup>6</sup> IU/litre)
- ofloxacin hydrochloride (60 mg/litre), and
- sulfamethoxazole (0.2 g/litre)

Dispense 1.0–2.0 ml of transport medium into sterile plastic screw-cap vials (Cryovials). It is best to store these vials at –20 °C until used. However, they can be stored at +4 °C for 48–96 hours (optimally less than 48 hours) or at room temperature for short periods of 1–2 days.

**Note:** Normal saline (NS) solution should not be used as a VTM. Adding BSA and antibiotics to NS changes the pH and this will destroy viruses.

# 2-Supplies



# Container

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



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# 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.

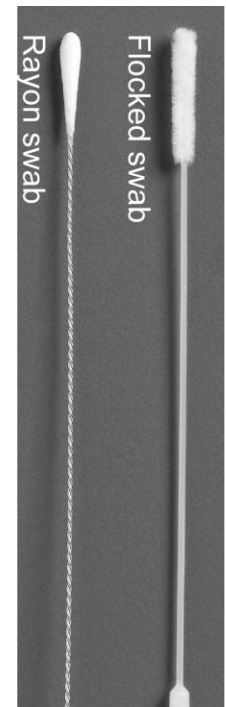
Swap tips:

**1- head:**

- Cotton
- rayon
- Nilon
- Metal

**2- stick:**

Woden  
Plastic  
metal



# Syringe

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



# Blood tube

- Blood collection tubes containing anticoagulant (ACD)



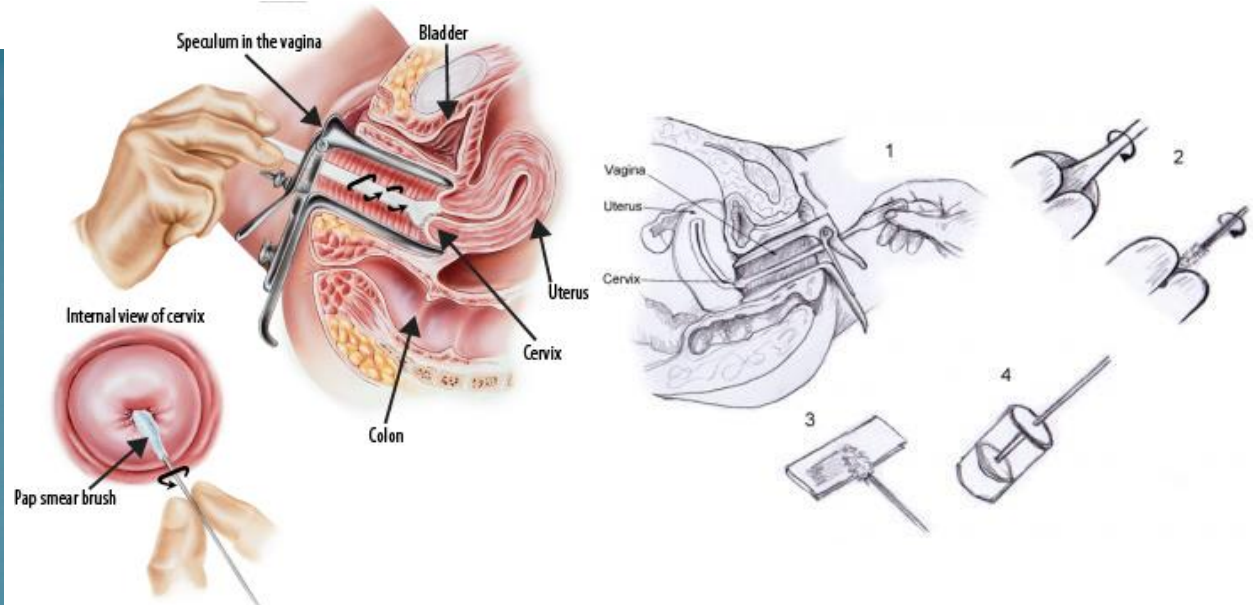
# 3-Equipment

# 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.



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# Surgical tools



# C-How to collect samples

Refer to the following information when selecting appropriate specimens for testing.

## **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.

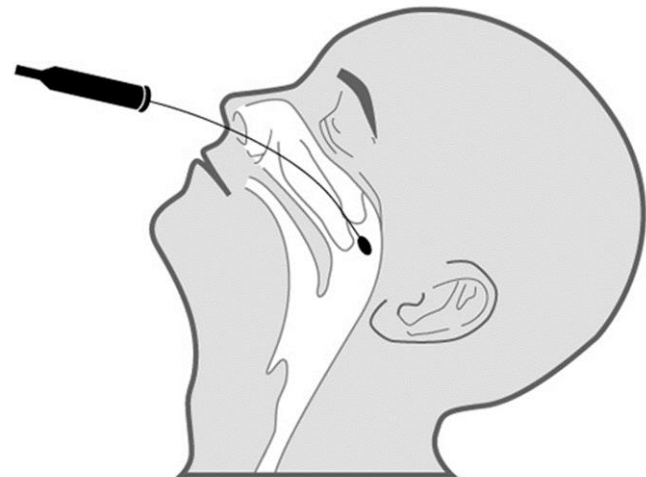


**Ocular swab:** Collect from lower conjunctiva using a swab moistened with sterile saline. Place swab in 1-2 ml of sterile saline or viral transport media.



**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.

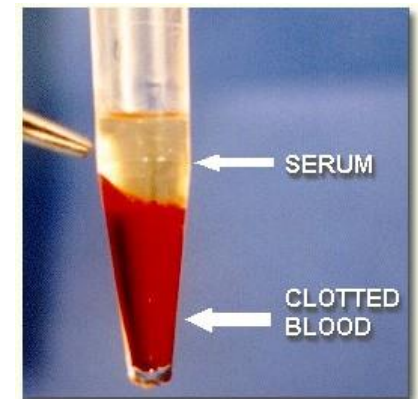
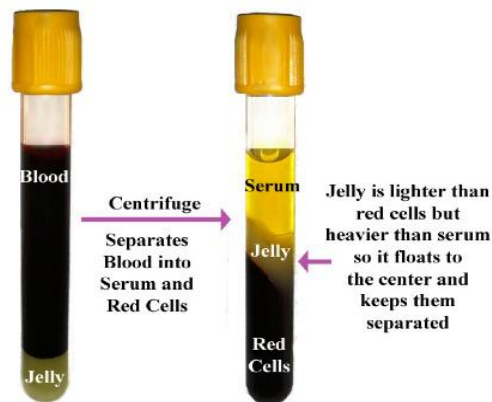


## 2-Blood (BLD):

**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.

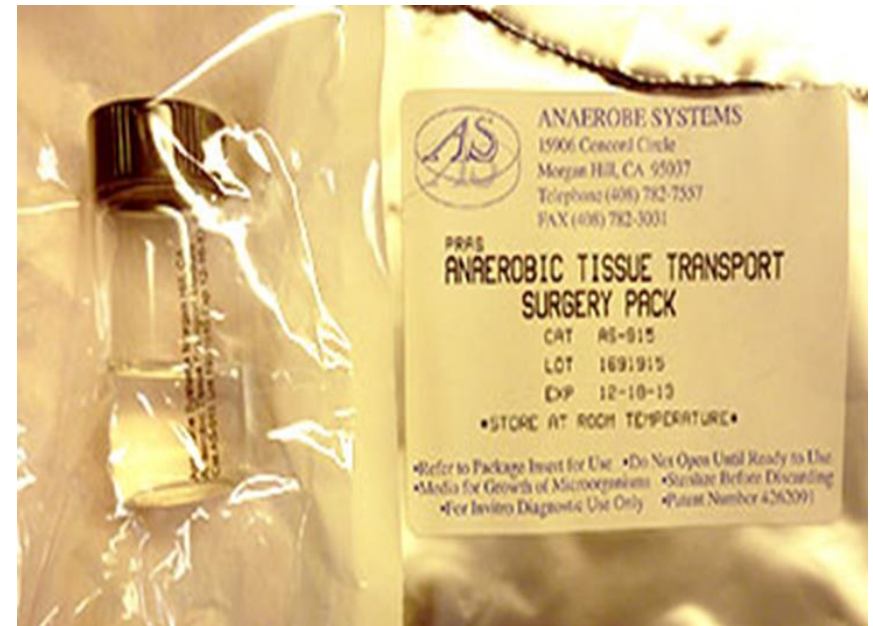
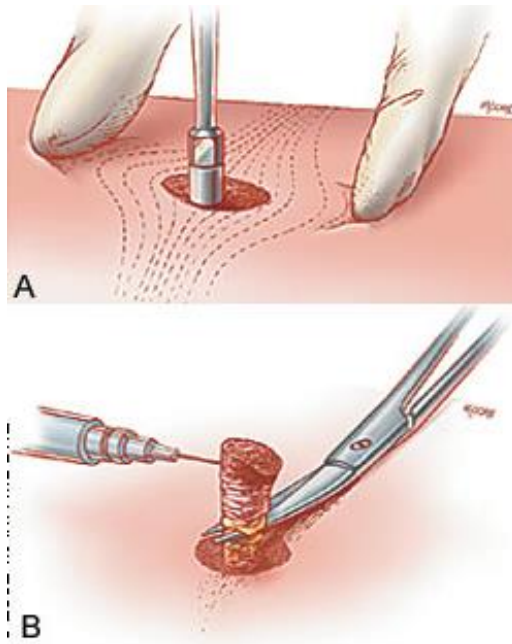


**2- Throat Aspirate**

**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



# D- Appropriate Specimens for Virus Culture

<b>Organ system involved</b>	<b>Agent suspected</b>	<b>Appropriate specimens</b>
Respiratory (upper respiratory, tracheobronchitis, pneumonitis)	rhinovirus, RSV influenza, parainfluenza  cytomegalovirus adenovirus, enterovirus	NP, BAL, OTHER RESP FLD, NW NP, BAL, LUNG BX, NW  BAL, LUNG BX, BLD, THRT THRT, BAL, STOOL
Central Nervous System (encephalitis, encephalomyelitis, meningitis, neuritis, polyradiculo-encephalopathy)	enterovirus cytomegalovirus	THRT, STOOL, CSF CSF, BLD
Skin, Mucous Membrane, Eye	varicella-zoster verpes simplex enterovirus adenovirus	VESICL VESICL, THRT, EYE SWB VESICL, THRT, STOOL EYE SWB
Urogenital	herpes simplex adenovirus enterovirus cytomegalovirus chlamydia	GENSWB, URN THRT, URN THRT, URN, STOOL URN, BLD, THRT GENSWB, URN
Gastrointestinal	adenovirus enterovirus cytomegalovirus herpes simplex	STOOL, THRT, BX  STOOL, BX, BLD STOOL, BX, BLD
Cardiovascular (Pericarditis, myocarditis)	influenza enterovirus	NP, NW STOOL, THRT, PERICD
Mononucleosis	cytomegalovirus	BLD, THRT, URN



## 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



16006 501 010 8



GFQ890W DFU-ID:43890

Please fill out patient data clearly in block letters.

**Patient data**

Family name \_\_\_\_\_

First name \_\_\_\_\_ Day Month Year \_\_\_\_\_

Id. No. \_\_\_\_\_

Physician \_\_\_\_\_  male  female

**Client data**

Clinical comments only (write clearly) \_\_\_\_\_

**Specimen data**

Sampling date \_\_\_\_\_ Time \_\_\_\_\_  
Day Month Year

**Sample type**

Serum (S)  EDTA pl. (EP)  CSF  Smear (SM)  
 EDTA bl. (EB)  Hepatit. (HP)  Erythrocytes (E)  Swab (SW)  
 Hepatit. (HB)  Citrate pl. (CP)  Blood card (BC)  Tissue (T)  
 Citrate bl. (CB)  Amniotic Fluid (AF)  Urine (U)

if relevant: w.o.g.  week  day

Faeces (F) 24 hour weight \_\_\_\_\_  Other (state) \_\_\_\_\_

Urine (U) 24 hour volume \_\_\_\_\_

Patient weight \_\_\_\_\_  No. of tubes sent \_\_\_\_\_

Patient height \_\_\_\_\_

Analyses required (please note sample requirements in the Test List)			
<input type="checkbox"/> E: 17-OH-Progesterone	S *	<input type="checkbox"/> E: Chlamydia trach. DNA	SWO
<input type="checkbox"/> E: ACE	S	<input type="checkbox"/> E: Citrate	U
<input type="checkbox"/> E: Acetylreceptor abs.	S	<input type="checkbox"/> E: CMV abs.	S
<input type="checkbox"/> E: ACTH, intact	EP *	<input type="checkbox"/> E: Cortisol	UO
<input type="checkbox"/> E: ADH + Osmolality	EP *	<input type="checkbox"/> E: Cortisol	S
<input type="checkbox"/> E: Albumin	UO	<input type="checkbox"/> E: Creatinine	S
<input type="checkbox"/> E: Aldosterone	S *	<input type="checkbox"/> E: Creatinine	UO
<input type="checkbox"/> E: Aluminium	S	<input type="checkbox"/> E: CRP	S
<input type="checkbox"/> E: Amino acid, quant.	EP *	<input type="checkbox"/> E: CRP ultrasensitive	S
<input type="checkbox"/> E: Amino acid, quant.	UO	<input type="checkbox"/> E: DHEA-S	S *
<input type="checkbox"/> E: ANA	S	<input type="checkbox"/> E: DNA abs. (ds) IgG	S
<input type="checkbox"/> E: ANCA-C	S	<input type="checkbox"/> E: Drug screen	U
<input type="checkbox"/> E: ANCA-P	S	<input type="checkbox"/> E: Drug confirmatory analysis	U
<input type="checkbox"/> E: Androstendione	S *	<input type="checkbox"/> E: EBV abs.	S
<input type="checkbox"/> E: Antithrombin	CP *	<input type="checkbox"/> E: ENA abs.	S
<input type="checkbox"/> E: β-2-Microglobulin	S *	<input type="checkbox"/> E: Endomysium IgA abs.	S
<input type="checkbox"/> E: Bite acids, total	S	<input type="checkbox"/> E: Endomysium IgG abs.	S
<input type="checkbox"/> E: C-3-comp.	S	<input type="checkbox"/> E: Factor VIII act.	CP *
<input type="checkbox"/> E: C-4-comp.	S	<input type="checkbox"/> E: Factor VIII assoc. ag.	CP *
<input type="checkbox"/> E: CA 125	S	<input type="checkbox"/> E: Factor VIII rist. co. fac.	CP *
<input type="checkbox"/> E: CA 15-3	S	<input type="checkbox"/> E: Ferritin	S
<input type="checkbox"/> E: CA 19-9	S	<input type="checkbox"/> E: Folate	EB
<input type="checkbox"/> E: C-Peptide	S *	<input type="checkbox"/> E: Folic acid	S *
<input type="checkbox"/> E: Cardiolipin abs.	S	<input type="checkbox"/> E: Free Testosterone	S *
<input type="checkbox"/> E: Carnitine, free	S *	<input type="checkbox"/> E: FSH	S
<input type="checkbox"/> E: Carnitine, total	S *	<input type="checkbox"/> E: FT3	S
<input type="checkbox"/> E: Catecholamines	EP *	<input type="checkbox"/> E: FT4	S
<input type="checkbox"/> E: Catecholamines	UO	<input type="checkbox"/> E: G-6-PD	EB
<input type="checkbox"/> E: CBC (Haemogram)	EB	<input type="checkbox"/> E: Galactosaemia screen	BC
<input type="checkbox"/> E: CDT	S	<input type="checkbox"/> E: Gastrin	S *
<input type="checkbox"/> E: CCP abs.	S	<input type="checkbox"/> E: Gliadin abs.	S
<input type="checkbox"/> E: Ceruloplasmin	S	<input type="checkbox"/> E: Haemoglobin A1c	EB
<input type="checkbox"/> E: Chlamydia pneum. abs.	S	<input type="checkbox"/> E: Haemoglobin electrop.	HB
<input type="checkbox"/> E: Chlamydia trach. abs.	S	<input type="checkbox"/> E: HBV-DNA qual.	EB
<input type="checkbox"/> E: Chlamydia trach. antigen	SWO	<input type="checkbox"/> E: HBV-DNA quant.	EB
<input type="checkbox"/> E: Chlamydia trach. DNA	S *	<input type="checkbox"/> E: HBV genotyping	EB
<input type="checkbox"/> E: ACE	S	<input type="checkbox"/> E: HBs abs.	S
<input type="checkbox"/> E: Acetylreceptor abs.	S	<input type="checkbox"/> E: HBs antigen	S
<input type="checkbox"/> E: ACTH, intact	EP *	<input type="checkbox"/> E: HCV abs.	S
<input type="checkbox"/> E: ADH + Osmolality	EP *	<input type="checkbox"/> E: HCV genotyping	EB
<input type="checkbox"/> E: Albumin	UO	<input type="checkbox"/> E: HCV-RNA qual.	EB
<input type="checkbox"/> E: Aldosterone	S *	<input type="checkbox"/> E: HCV-RNA quant.	EB
<input type="checkbox"/> E: Aluminium	S	<input type="checkbox"/> E: Helicobacter pylori abs.	S
<input type="checkbox"/> E: Amino acid, quant.	EP *	<input type="checkbox"/> E: Hepatitis screen	S
<input type="checkbox"/> E: Amino acid, quant.	UO	<input type="checkbox"/> E: Herpes profile	S
<input type="checkbox"/> E: ANA	S	<input type="checkbox"/> E: Herpes simplex virus abs.	S
<input type="checkbox"/> E: ANCA-C	S	<input type="checkbox"/> E: HGH	S *
<input type="checkbox"/> E: ANCA-P	S	<input type="checkbox"/> E: Histology	U
<input type="checkbox"/> E: Androstendione	S *	<input type="checkbox"/> E: HIV 1/2 abs.	S
<input type="checkbox"/> E: Antithrombin	CP *	<input type="checkbox"/> E: HIV-1 RNA quant.	EB
<input type="checkbox"/> E: β-2-Microglobulin	S *	<input type="checkbox"/> E: HLA B27	S
<input type="checkbox"/> E: Bite acids, total	S	<input type="checkbox"/> E: HLA Typing (A, B + C)	HB+EB
<input type="checkbox"/> E: C-3-comp.	S	<input type="checkbox"/> E: HLA Typing (A, B, C, DG + DR)	HB+EB
<input type="checkbox"/> E: C-4-comp.	S	<input type="checkbox"/> E: Homocysteine	HB+EB
<input type="checkbox"/> E: CA 125	S	<input type="checkbox"/> E: IBC, total	S
<input type="checkbox"/> E: CA 15-3	S	<input type="checkbox"/> E: IgE	S
<input type="checkbox"/> E: CA 19-9	S	<input type="checkbox"/> E: IGF-1	S
<input type="checkbox"/> E: C-Peptide	S *	<input type="checkbox"/> E: Immunofixation	S
<input type="checkbox"/> E: Cardiolipin abs.	S	<input type="checkbox"/> E: Insulin	S *
<input type="checkbox"/> E: Carnitine, free	S *	<input type="checkbox"/> E: Lead	HB
<input type="checkbox"/> E: Carnitine, total	S *	<input type="checkbox"/> E: LH	S
<input type="checkbox"/> E: Catecholamines	EP *	<input type="checkbox"/> E: Lupus anticoag. / inhibitor	CP *
<input type="checkbox"/> E: Catecholamines	UO	<input type="checkbox"/> E: Measles abs.	S
<input type="checkbox"/> E: CBC (Haemogram)	EB	<input type="checkbox"/> E: Metazephrine + Normetazephrine	UO
<input type="checkbox"/> E: CDT	S	<input type="checkbox"/> E: Microsomal abs. (TPO)	S
<input type="checkbox"/> E: CCP abs.	S	<input type="checkbox"/> E: Mitochondriat abs.	S
<input type="checkbox"/> E: Ceruloplasmin	S	<input type="checkbox"/> E: Mycoplasma pneu. abs.	S
<input type="checkbox"/> E: Chlamydia pneum. abs.	S	<input type="checkbox"/> E: Neonatal screening	BC
<input type="checkbox"/> E: Chlamydia trach. abs.	S	<input type="checkbox"/> E: Oligoclonal banding	S+CSF
<input type="checkbox"/> E: Chlamydia trach. antigen	SWO	<input type="checkbox"/> E: Organic acids screen	U

**Other Analyses**

E: this is for internal lab. use only

\* must be sent FROZEN

Please refer to our Test List for further special requirements and/or guidelines for this test.

These labels are for the patient's tube(s). Those labels not needed - please tear off and discard - do not send to us.

<p>specimen material</p> <p>GFQ890W 1</p> <p>specimen material</p> <p>GFQ890W 2</p> <p>specimen material</p> <p>GFQ890W 3</p> <p>specimen material</p> <p>GFQ890W 4</p> <p>specimen material</p> <p>GFQ890W Y</p> <p>specimen material</p> <p>GFQ890W 5</p>	<p>GFQ890W</p> <p>This label should be stuck onto the copy attached and kept for your records.</p> <p><b>DO NOT SEND TO US.</b></p> <p>GFQ890W DFU-ID:43890</p>
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**The request form**

# Educational Videos

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