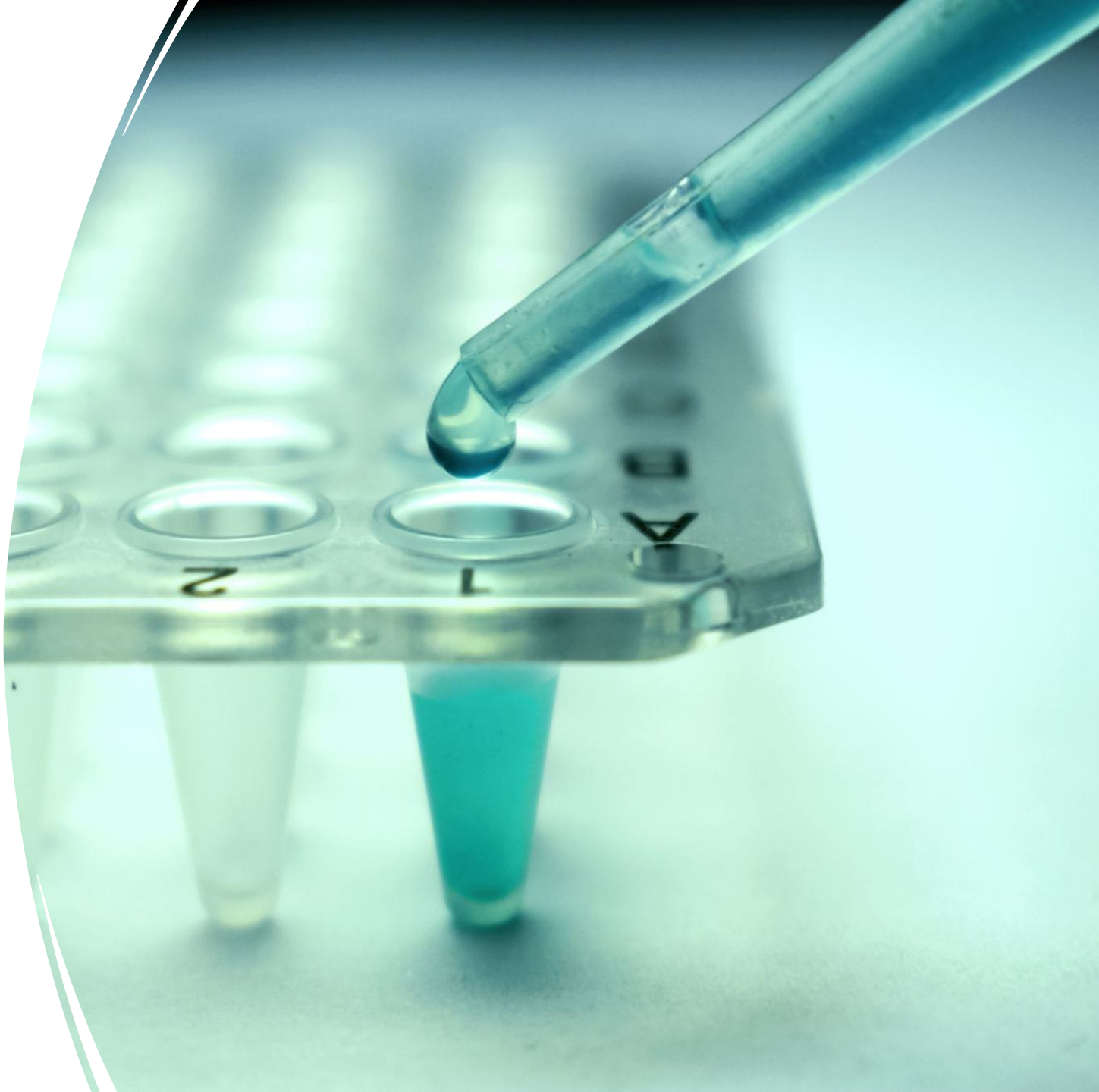
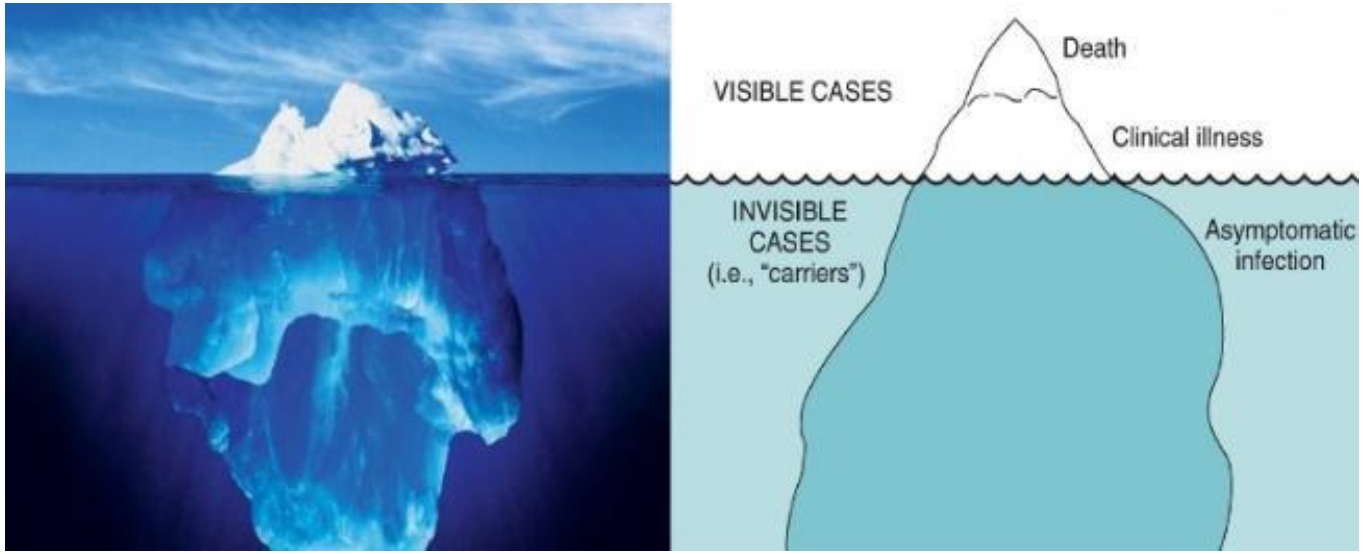

VIRAL DIAGNOSIS

- It is a collection of all clinical and laboratory applications to be performed for the diagnosis of viral infections.

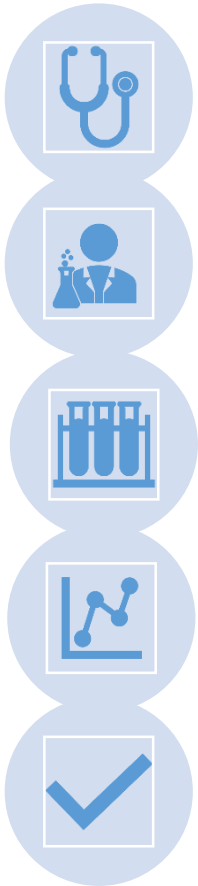




- Viruses can cause a wide range of diseases, from mild infections to very serious infections that can lead to death.
- Rapid and accurate detection of viral infections is of great importance
  - for disease limitation (especially in outbreaks, zoonoses and notifiable diseases),
  - prevention of losses,
  - determination of the necessary control/eradication methods in disease control,
  - Briefly, for proper disease management

https://www.docplexus.com/posts/iceberg-phenomenon-of-disease

The path to diagnosis includes the following steps:



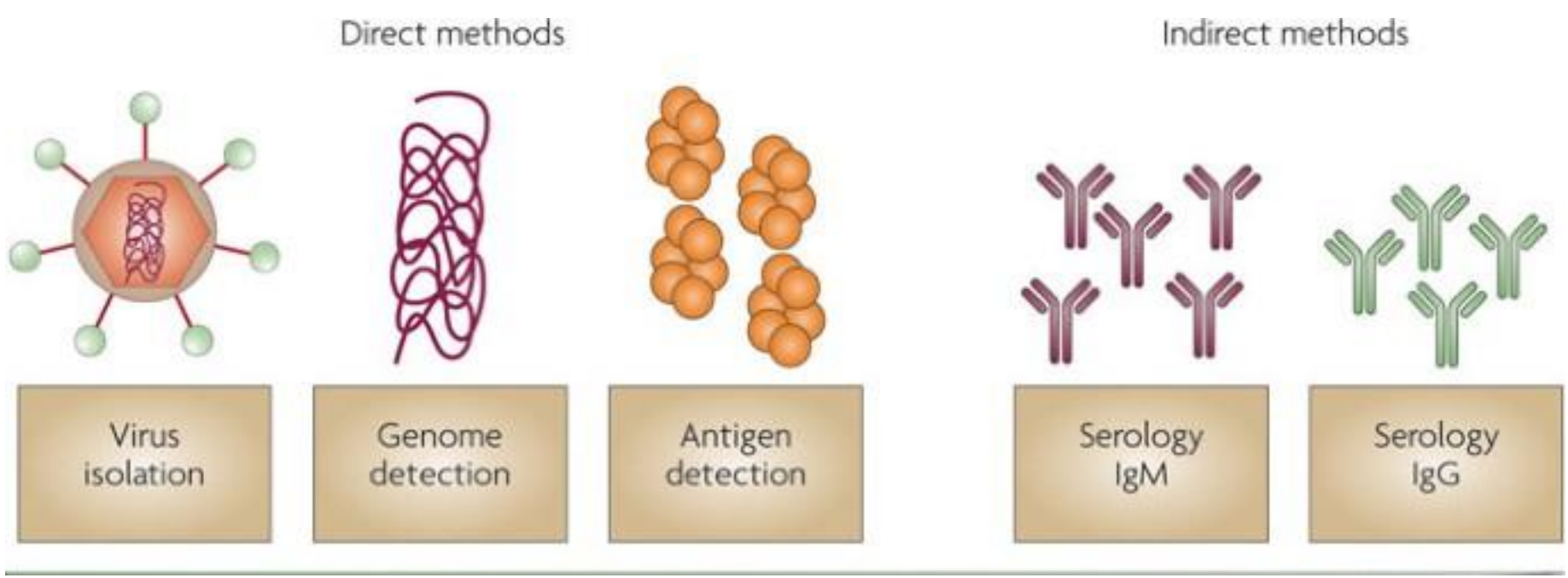
1. Clinical findings and evaluation of these findings
2. Laboratory tests (ordered by the physician)
3. Laboratory Assays and Results
4. Interpretation of results
5. DIAGNOSIS

1. Clinical findings and evaluation of these findings

- It includes the findings of the physical examination to be performed in accordance with the information (anamnesis) given by the animal (patient) owner.
- Certain suspected infections are noted as a result of the physical examination.
- Differential diagnosis of these infections is only possible with laboratory confirmation.

- Why are virologic diagnostic methods (laboratory diagnostic methods) important?
  - ➢Confirmation of clinical diagnosis
  - ➢Differential diagnosis of infections (differential diagnosis)
  - ➢Obtaining epidemiological data
  - ➢Prognosis and monitoring of infections
  - ➢Control/eradication
- The most important factors to be considered at this point, especially in terms of the samples to be taken and the technique to be used;
  - ✓Period of infection (acute, convalescent)
  - ✓Form of infection (generalized, local)

Accordingly, samples are taken from sick animals for DIRECT or INDIRECT diagnosis.



Peeling, R *et al.* https://doi.org/10.1038/nrmicro2459

Direct (Viral) diagnosis: Diagnosis is the process by which the virus are isolated, or viral structures (nucleic acid, protein sub-structures) detected.

Indirect Diagnosis: Diagnosis is made by serologic or biochemical detection of Ig that have been formed against viral infection after viral infection and also those that are released from degenerated or destroyed cells.

Any sample taken from an individual for diagnostic purposes is called a **Morbid (Diseased) material.**

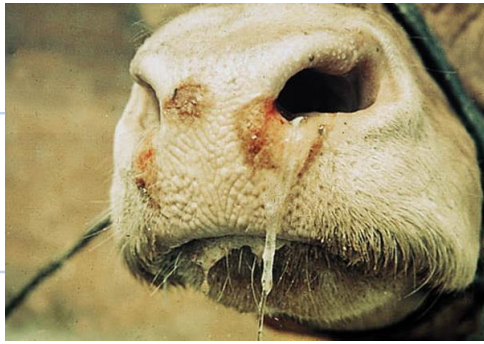
Specimens that can be taken for **direct diagnosis**

From live animals

- Blood from viremic animals (defibrinated & anticoagulated)

- Depending on the clinical course of infection, feces, urine, vesicle contents, skin scrapings,
- Swab (nasal, conjunctival, pharyngeal, genital, rectal)

- Body fluids (uterus, prepuce, rectum), milk, semen
- Gastric aspirates



- Puncture fluids (abscess contents, peritoneal or pleural effusions)

https://www.pennmedicine.org/updates/blogs/transplant-update/2016/april/monthly-blood-sample-process-for-kidney-patients

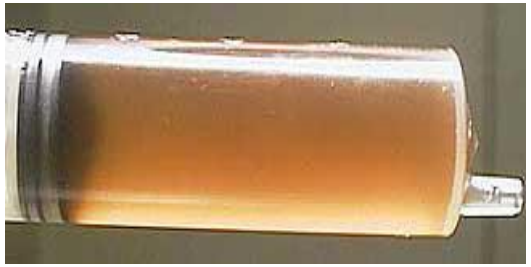
NADIS - National Animal Disease Information Service -

http://www.erewise.com

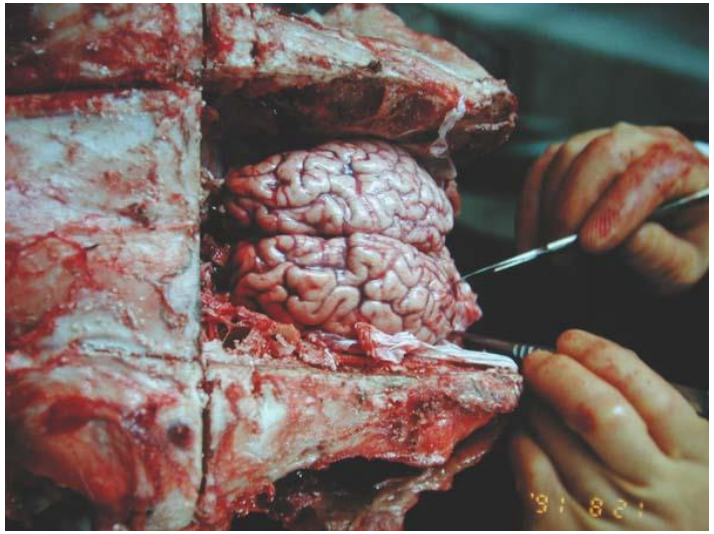
Specimens that can be taken for **direct diagnosis**

FROM DEAD or KILLED ANIMALS

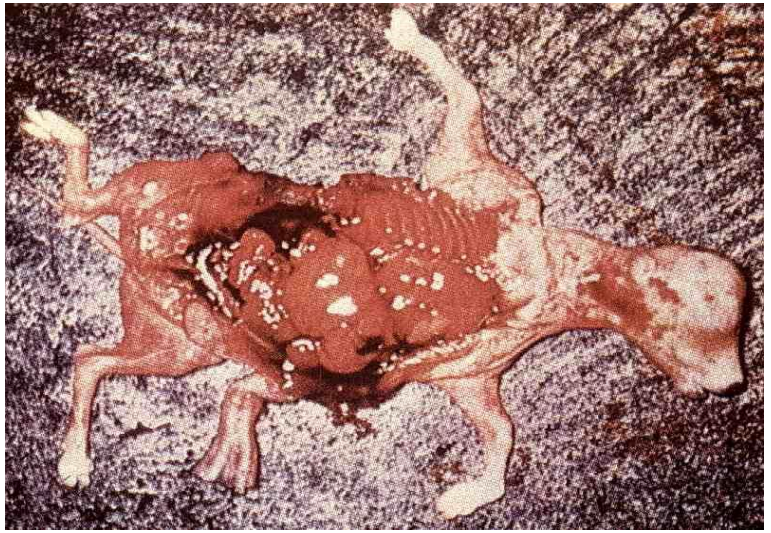
- All kinds of organ material,
- Fluids accumulated in the chest and abdominal cavity (effusions),
- Cardiac blood
- Intestinal contents



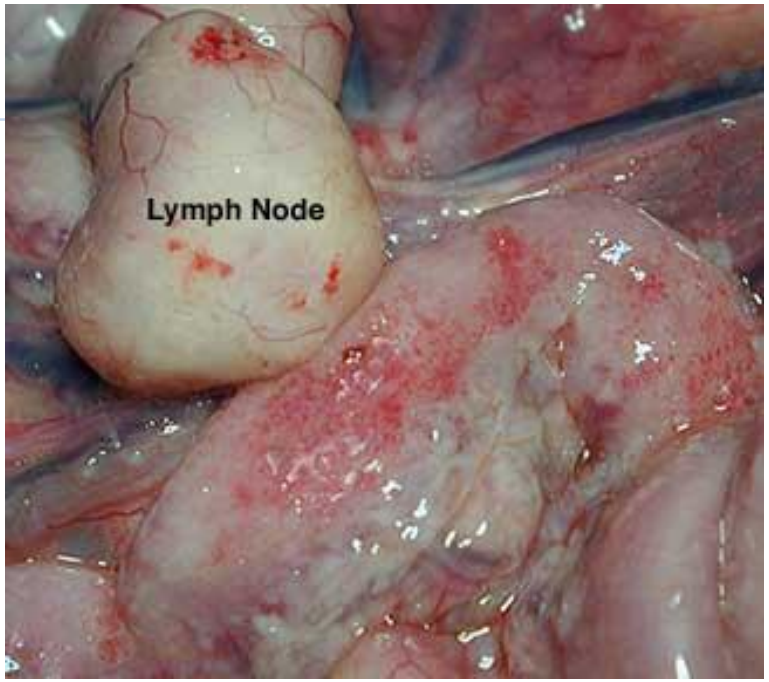
http://www.lbah.com/word/feline/feline-infectious-peritonitis-fip/



http://i227.photobucket.com/albums/dd65/flatuloman/BSE-Autopsy-Brain.jpg



http://ec2-107-21-65-169.compute-1.amazonaws.com/content/ABAAAAe3sAA/segredos-manejo-cocho-na-fazenda



http://www.lbah.com/word/feline/feline-infectious-peritonitis-fip/

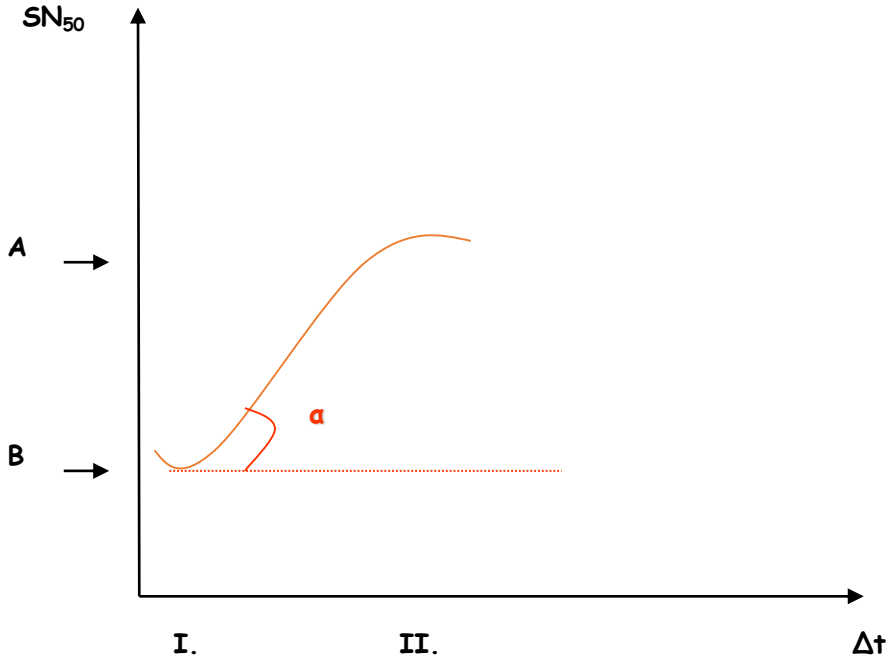
Generally,

Diseases with Respiratory Symptoms:	from live animals: Nasal, pharyngeal, oral swabs,
	from dead animals: lesioned lung tissue, mediastinal lymph node, tracheal scrape, effusion sample
Diseases with Digestive System Symptoms:	from live animals; feces, rectal swap
	from dead animals: esophageal, gastric and intestinal mucosa scrapings, tissue fragments, gastric and intestinal contents, mesenteric lymph nodes, peyer's plaques and ascites fluid if available.
Diseases with CNS Symptoms:	from live animals; CSF,
	from dead animals; CNS tissue.
Multisystemic Diseases:	from live animals; Blood, sample of relevant clinical finding above
	from dead animals: lymphoreticular tissue (spleen, liver, lymph nodes), pericardium, effusion and ascites fluids and all lesioned tissues.

SENT TO THE LABORATORY FOR VIRAL DIAGNOSIS!!!

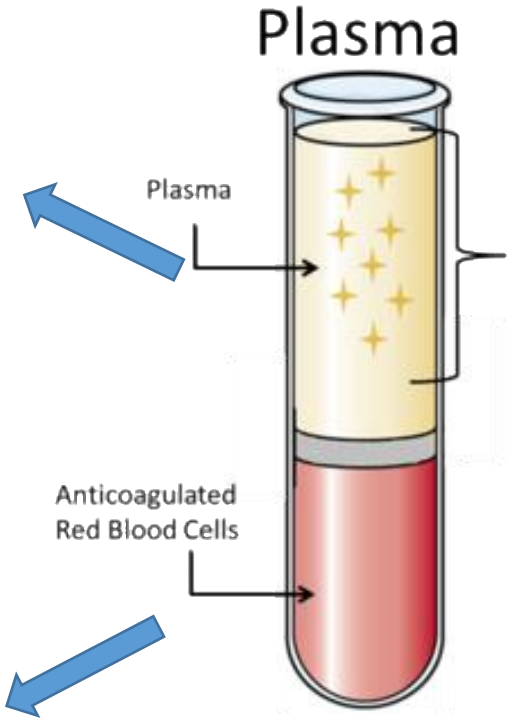
Specimens that can be taken for **indirect diagnosis**

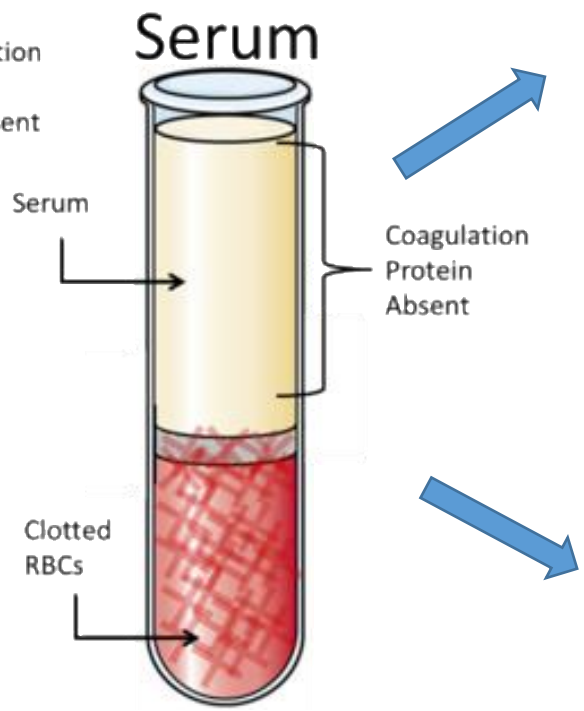
- Indirect diagnosis is mainly performed using blood serum samples from living or non-living animals.
- In addition to blood serum, serologic diagnostic procedures can also be widely applied to milk and CSF samples.

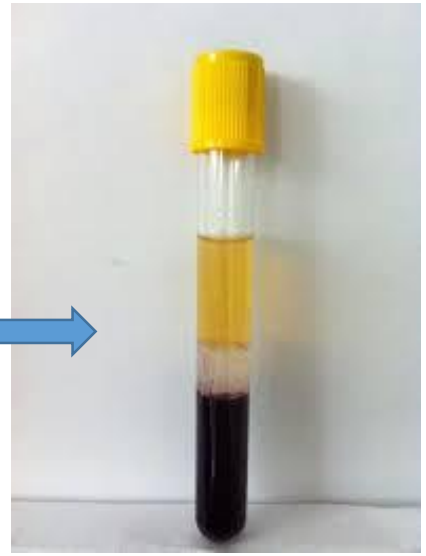


At least 2-fold antibody titer increase (B<2A) in two blood samples (I and II) taken 15 days apart is indicative of previous acute infection.

Blood Samples





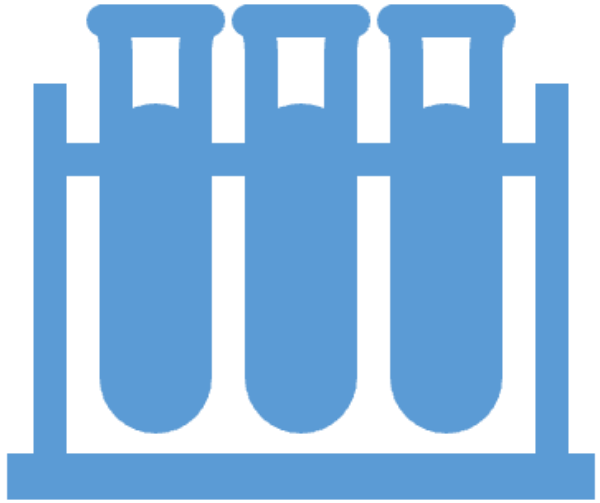


https://quizlet.com/92156986/clinlab-exam-1-lecture-3-collectionprocessing-blood-flash-cards/

Specimens that can be taken for **indirect diagnosis**

Biochemical and hematological parameters can also be used to provide important clues for the diagnosis of infections.

• Orthomyxovirus	NANA (N-acetylneuraminic acid)
• Hepatitis	Liver enzymes (ALP, AST, SGPT etc.)
• Parvovirus	low white blood cell count, leukopenia
• Bovine leukosis	Increased leukocyte count, leukocytosis



★★★

Whatever the purpose of the samples, they must be sent to the laboratory with a report. The report contains the following information;

- Name of the animal owner
- Name of the animal, Species/breed, Gender, Age
- Clinical symptoms of the disease
- Summary of treatment administered, etc.
- Pathological findings (if autopsy was performed)
- Suspected and wanted to be detected infectious agents
- which vaccinations, if any

Transportation of samples

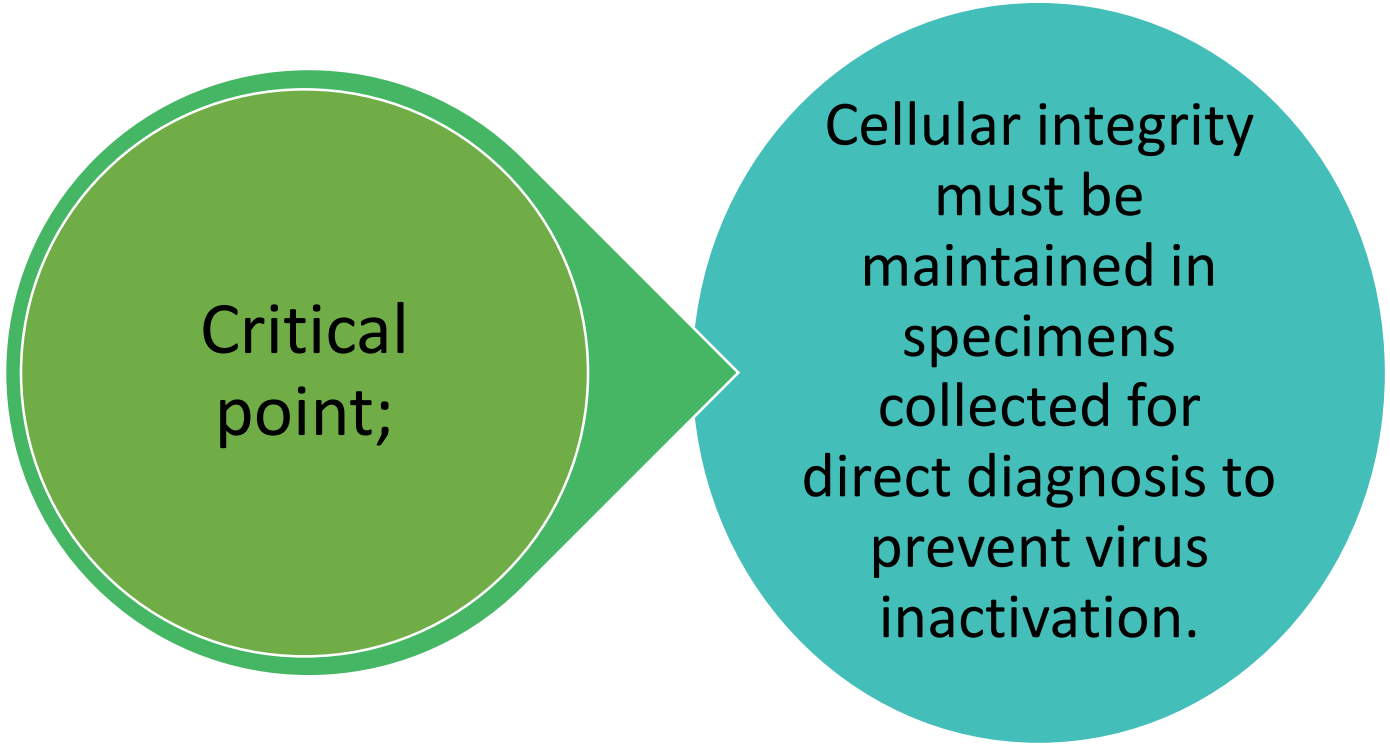
Samples must be sent to the laboratory without breaking the cold chain.

http://www.therapak.co.uk/catalog/insulated_shippers

http://www.ebay.com/itm/Techni-Ice-Standard-Use-2-Ply-Disposable-Minimum-Reuse-Dry-Ice-Pack-5-Sheets-/141663693404

http://urun.gittigidiyor.com/ev-bahce/buz-akusu-buz-kasedi-360-gr-75-adet-258456017

http://www.sogukzincirtasima.com/index.php?route=product/product&product_id=183



Transport fluids are used for this purpose;

- PBS (Phosphate buffered saline)
- PBS with 50% glycerin
- Serum Physiologic (0.9% isotonic sodium chloride (NaCl) solution)

formaldehyde - should never be used as a viral transport fluid!!!

CHOOSING THE RIGHT LABORATORY

- Samples collected should be sent to laboratories capable of testing for the pathogen causing the suspected disease;
- Institute Laboratories under the Ministry of Agriculture and Forestry
- University laboratories
- Private laboratories

Tablo 1. Enstitü Müdürlüklerinin sorumluluk alanına giren iller.

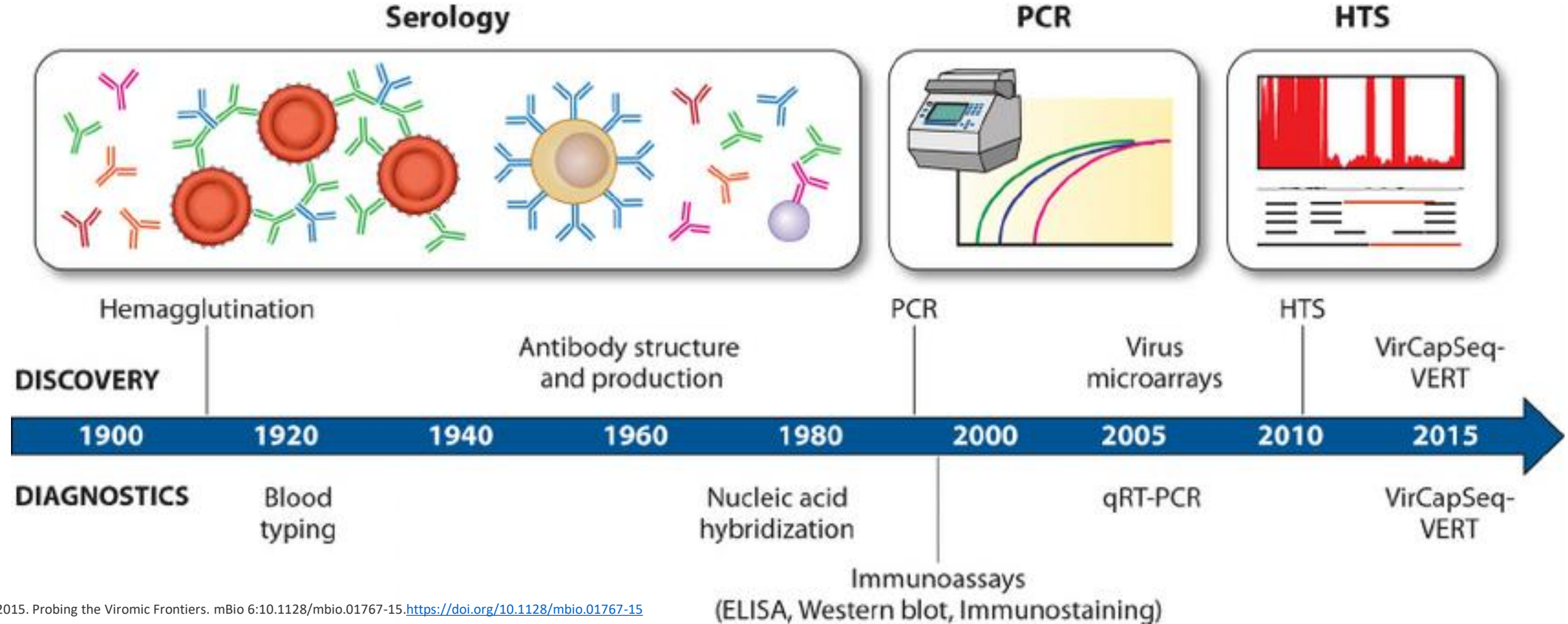
ENSTİTÜ ADI	İLLER
Veteriner Kontrol Merkez Araştırma Enstitüsü Müdürlüğü Etlik-ANKARA	Ankara, Bartın, Bolu, Çankırı, Çorum, Eskişehir, Karabük, Kastamonu, Kayseri, Kırıkkale, Kırşehir, Nevşehir, Yozgat, Zonguldak ve bazı analizlerde tüm iller.
Pendik Veteriner Kontrol Enstitüsü Müdürlüğü İSTANBUL	Balıkesir, Bilecik, Bursa, Çanakkale, Düzce, Edirne, İstanbul, Kırklareli, Kocaeli, Sakarya, Tekirdağ, Yalova.
Bornova Veteriner Kontrol Enstitüsü Müdürlüğü İZMİR	Aydın, Denizli, İzmir, Kütahya, Manisa, Muğla, Uşak.
Konya Veteriner Kontrol Enstitüsü Müdürlüğü	Afyonkarahisar, Aksaray, Antalya, Burdur, Isparta, Karaman, Konya, Niğde.
Adana Veteriner Kontrol Enstitüsü Müdürlüğü	Adana, Adıyaman, Gaziantep, Hatay, Kahramanmaraş, Kilis, Mersin, Osmaniye, Şanlıurfa.
Samsun Veteriner Kontrol Enstitüsü Müdürlüğü	Amasya, Giresun, Ordu, Rize, Samsun, Sinop, Sivas, Tokat, Trabzon.
Erzurum Veteriner Kontrol Enstitüsü Müdürlüğü	Ağrı, Ardahan, Artvin, Bayburt, Erzincan, Erzurum, Gümüşhane, Iğdır, Kars.
Elazığ Veteriner Kontrol Enstitüsü Müdürlüğü	Batman, Bingöl, Bitlis, Diyarbakır, Elazığ, Hakkari, Malatya, Mardin, Muş, Siirt, Şırnak, Tunceli, Van.
Şap Enstitüsü	Tüm İller

2020/01 Sayılı Hayvan Hastalıkları ile Mücadele ve Hayvan Hareketleri Kontrolü Genelgesi https://vetkontrol.tarimorman.gov.tr/merkez

CHOOSING THE RIGHT LABORATORY METHOD:

- ➢Samples taken (type, quality, etc.)
- ➢Suspected diseases

- Techniques recommended by the World Organization for Animal Health (WOAH)



asmussen AL. 2015. Probing the Viromic Frontiers. mBio 6:10.1128/mbio.01767-15.https://doi.org/10.1128/mbio.01767-15

Preparation of Inoculum from Morbid/Diseased material

- Morbid/Diseased material: Any sample taken from individuals (living or dead) suspected of having a virus infection is called morbid material.
- Inoculum: The morbid/diseased material that has been prepared according to its structure, made ready for inoculation into cell cultures and cleared of all kinds of unicellular microorganisms is called inoculum.

Morbid/Diseased material vs Inoculum

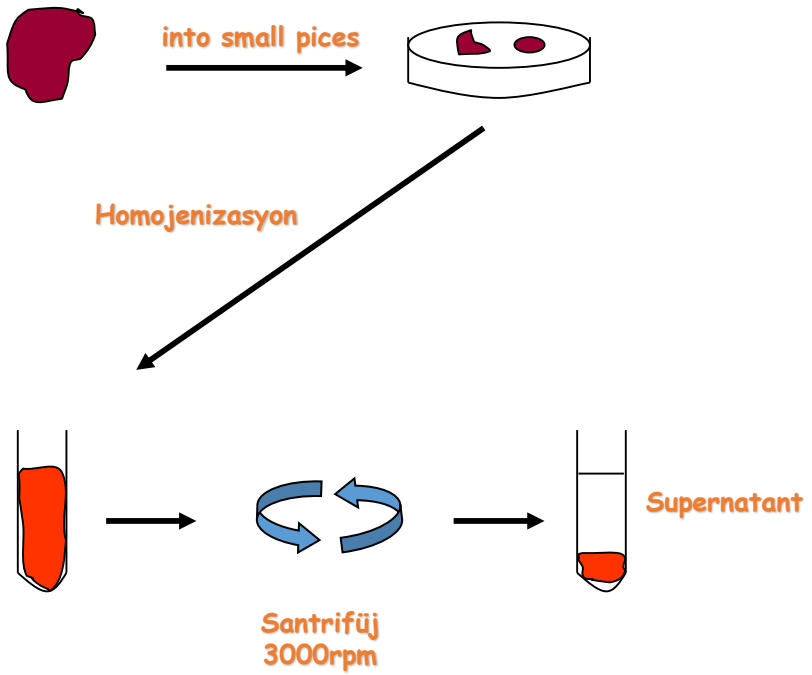
- The main difference between the two concepts is the partial sterilization of the sample from non-viral pathogens before inoculation into cell culture systems.

This is mandatory!!!!!!

- The purification of the processed sample from non-viral pathogens is performed in the following steps
  - Addition of concentrated antibiotics (Pen, Strep, Kana)
  - Filtration (cellulose acetate filters with 0.22-0.45 µm pore size)
  - High speed centrifugation (15000-20000rpm)
  - Lipid melting treatment

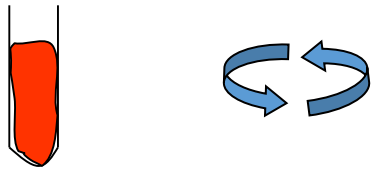
1. Organ Material

1. Organ Surface Disinfection
2. The process involves taking a certain amount from the organ and cutting the received piece into small pieces.
3. The small pieces are then resuspended in 1:10 ratio with PBS and homogenized.
4. The resulting mixture is then centrifuged at 3000 rpm.
5. The supernatant is collected and stored.
6. Sterilization with the addition of antibiotics,
7. Then agar inoculation, and control for bacteria (don't want bacteria),
8. Inoculation into cell culture or storage at -80°C.

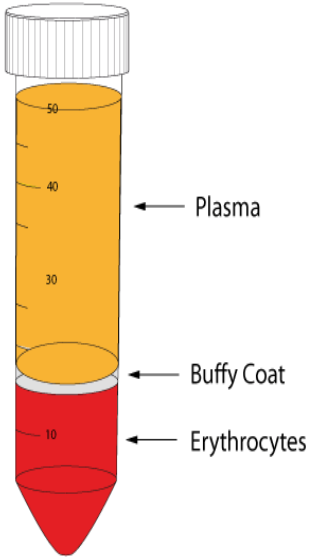


2. Blood (For Virus Isolation)

1. Blood is taken into an anticoagulated (EDTA etc.) tube.
2. Centrifuge at 1500-2000rpm for 10 minutes at 4°C.
3. The central layer of leukocyte is collected with a capillary pipette and resuspended in 2-3 ml PBS.
4. Centrifuge under the same conditions.
5. Leukocytes are collected again. This process is repeated 2-3 times.
6. Leukocytes taken from the last wash are either immediately inoculated into the cell or frozen by adding cryopreservative (DMSO).

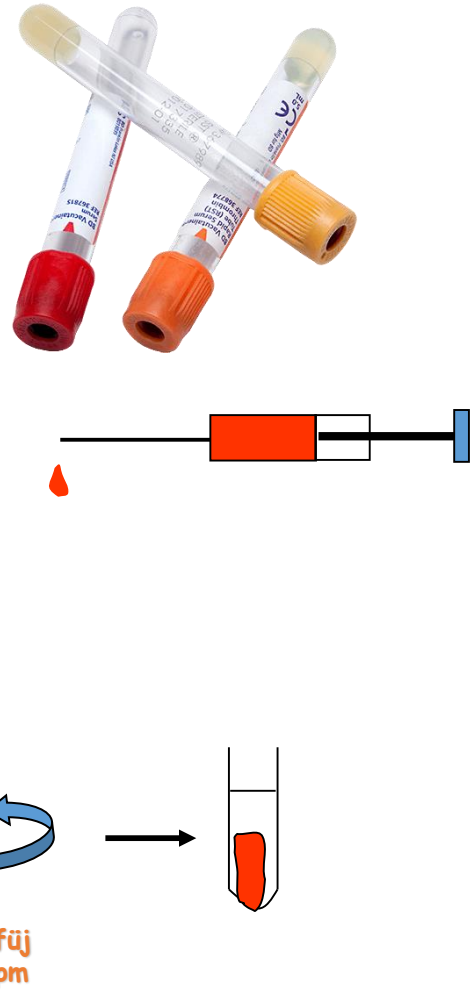


Santrifüj
2000rpm



2. Blood (for Serological Diagnosis)

1. Blood is collected in an unadulterated and clean glass tube or a tube containing gel or kaolin.
2. After coagulation, separate the clot from the tube wall with a wire.
3. then centrifuged for 10 minutes at 2000rpm at 4°C.
4. The serum obtained is transferred to a clean tube.
5. The sample should be inactivated at 56°C for 30 minutes prior to use.
6. If it is not intended for immediate use, it should be frozen at -20°C.



3. Fecal samples

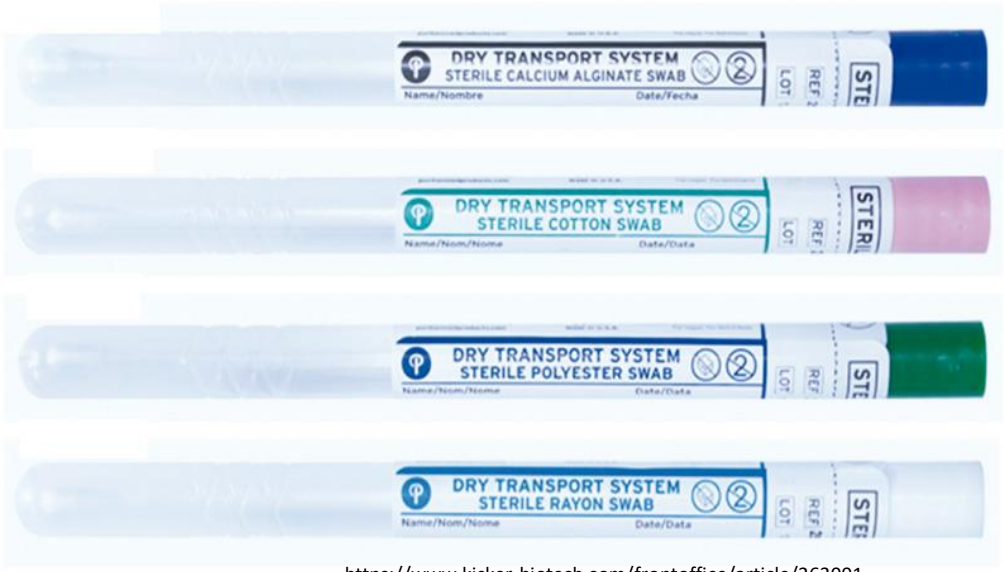
1. Dilution 1/10 in PBS with antibiotic and homogenization.
2. Centrifugation at 3000 rpm
3. Upper part (supernatant) is collected, sterilization with the addition of antibiotics.
4. Then agar inoculation, and control for bacteria (don't want bacteria),
5. Inoculation into cell culture or storage at -80°C.

http://www.livepathlab.com/facilities.php

http://www.medicaldaily.com/fresh-or-frozen-fecal-transplants-are-effective-combatting-c-difficile-infection-369108

4. Swabs

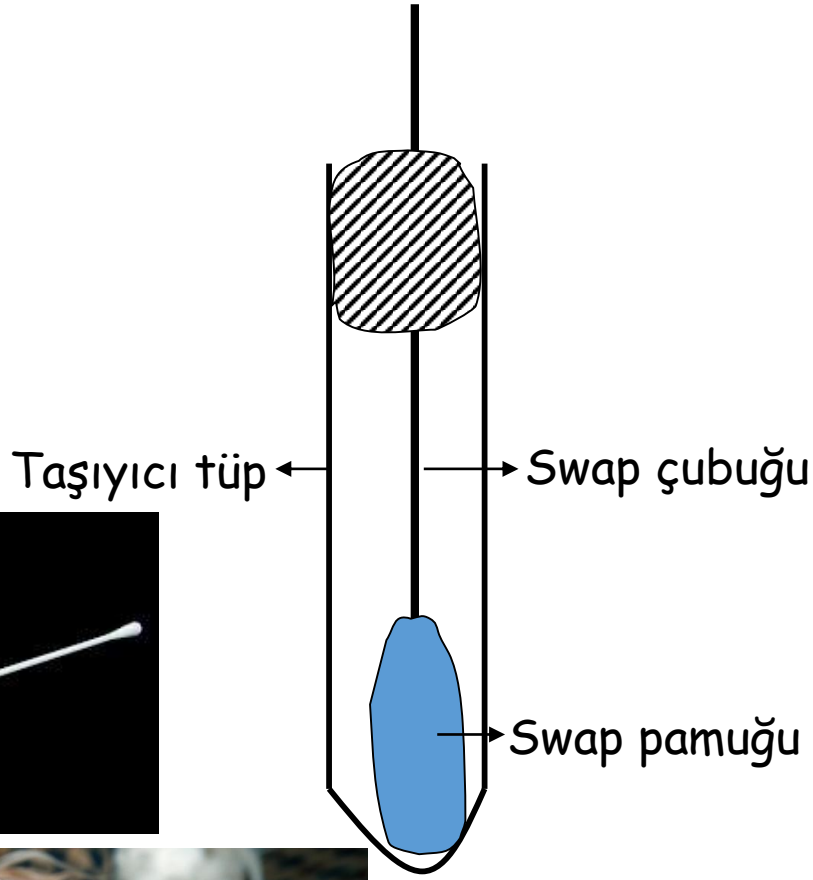
- Furthermore, swabs destined for PCR analysis **should not be placed in agar or charcoal-based transport media**. Calcium alginate swabs should be avoided.
- Instead, cotton or dacron swabs should be shipped in a tube with a few drops of sterile saline or viral transport media.

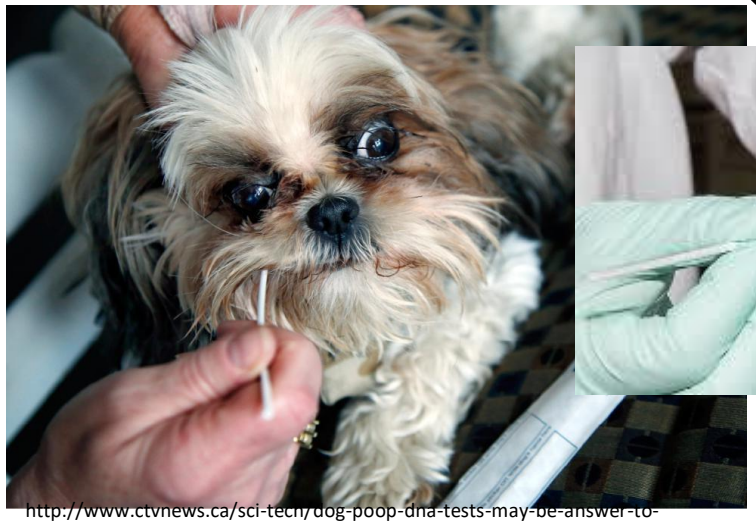


https://www.kisker-biotech.com/frontoffice/article/263001

4. Swabs

1. Samples arriving at the laboratory are vortexed in 2-3 ml of antibiotic-containing PBS.
2. The cotton is squeezed on the tube wall and the cotton is discarded.
3. The liquid is centrifuged at 3000rpm for 10 minutes.
4. Antibiotics are added, then agar inoculation, and control for bacteria (don't want bacteria),
5. Inoculation into cell culture or storage at -80°C.





http://www.ctvnews.ca/sci-tech/dog-poop-dna-tests-may-be-answer-to-discourteous-pet-owners-1.1562654

PRODUCTION OF VIRUSES IN CELL CULTURE

Why do we cultivate viruses in cell culture?

- To isolate and identify them for the diagnosis of viral infections,
- to study pathogenesis
- To obtain and standardize prophylactic products
- For use in serological tests

For the success of isolation:

- Samples are taken at the right time
- Appropriate sample collection
- Delivery of samples to the laboratory under appropriate conditions.
- Using cells appropriate for the virus to be isolated

Virus Inoculation

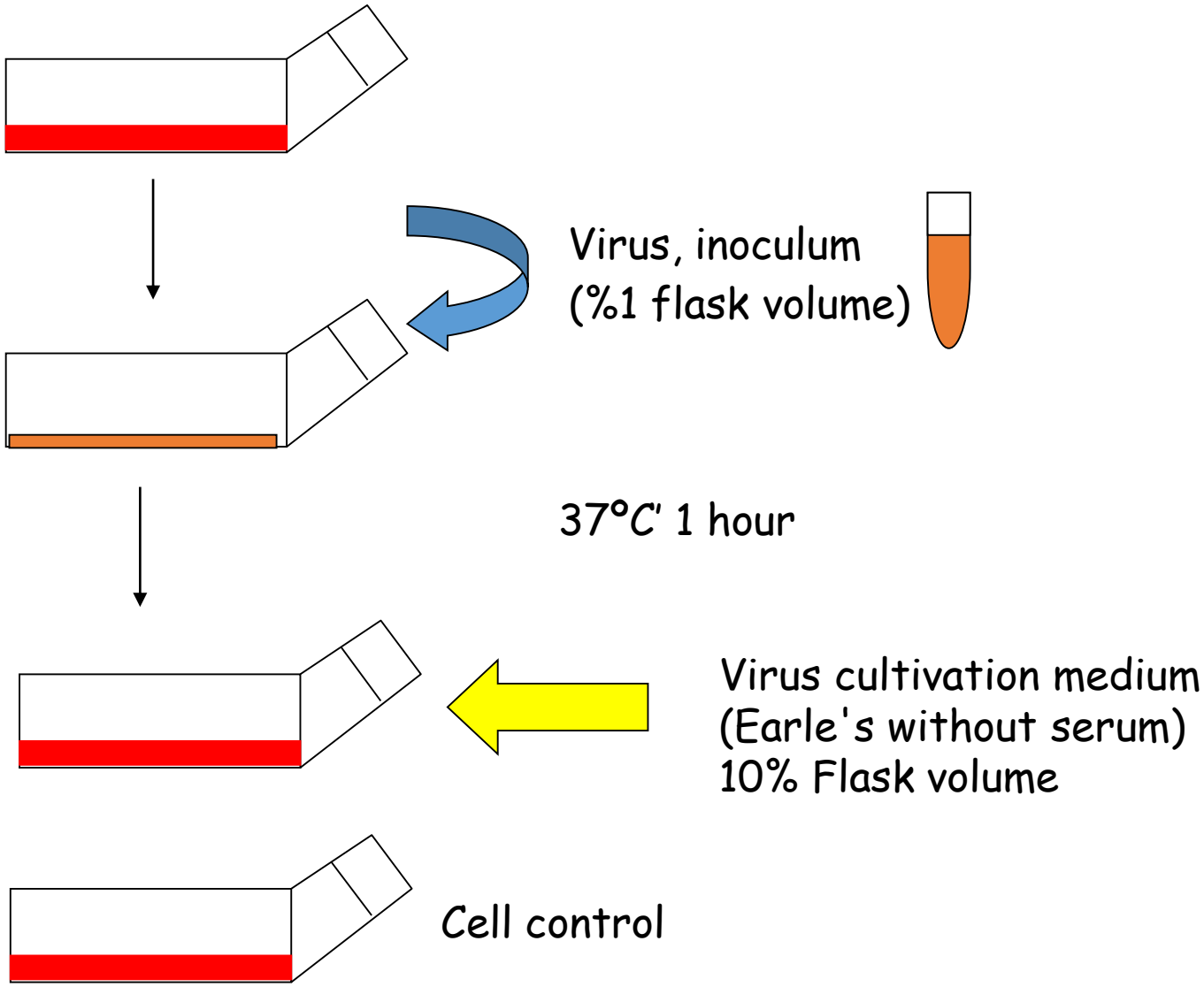
Adsorption Technique:

- Following virus inoculation, there is an additional incubation period of 1 hour at 37^{o}C.

Non-adsorption technique:

- After virus inoculation, the virus production medium is placed without any additional waiting time.

Adsorption Technique:



Non-Adsorption Technique: