Role of rapid and specific Laboratory diagnosis in the efficiency of contagious diseases control

Infectious diseases of livestock

Major economic losses

Significance zoonoses

Timely and accurate diagnosis is critical to the global efforts to prevent and treat infectious diseases.

A necessary step

Establishment of a good diagnostic laboratory

Rapid and Reliable diagnosis

Proper preventive and control measures

To be useful

Confirmatory Diagnostic methods must be

Accurate *Simple* *Affordable* *Fast*

Sample

Laboratory investigation critically dependent on the quality and appropriateness of the specimens collected for analysis.

Why we have to run early diagnosis?

- Effective control measures and block transmission of the infectious agent to other animals or man if zoonotic.
- Preventing the development of long-term complications.

Why?

- Screening for asymptomatic carriers;
- Detection of pre or post infection active subclinical infection, which is essential for control of the disease at the farm on national or international level.
- **Disease surveillance; it is the practice of disease case reporting**.

- **Epidemiological studies (for example, rapid assessments of disease burden or outbreak investigations.**
- Detecting infections with markers of drug resistance.

- Reducing unnecessary diagnostic testing and treatment.
- Start effective treatment.
- Isolate, identify, and determine the antimicrobial susceptibility pattern

- Differentiate vaccinated from non-vaccinated (infected) animals.
- To track source and place of foodborne disease-causing bacteria across countries and continents.

To achieve all these

New more specific more sensitive diagnostic tests are required.

The basic performance characteristics of a test designed to distinguish infected from uninfected individuals are

Sensitivity the probability that a truly infected individual will test positive,

specificity the probability that a truly uninfected individual will test negative. These measures are usually expressed as a percent

How to make a diagnosis?

- Traditional Phenotypic- Microscopic and macroscopic morphology and biochemical identification.
- Immunological- Serological analysis.
- Genotypic- Genetic technique.

❖ Conventional diagnostic tests

Culture …Remains the gold standard for identifying organisms, allow antimicrobial susceptibilities to be determined; not all pathogens can be cultured, making alternative tests necessary. Besides it is mostly time consuming

Automated identification systems

Today; the use either commercially available miniaturized biochemical test systems or automated instruments for biochemical tests and for susceptibility testing.

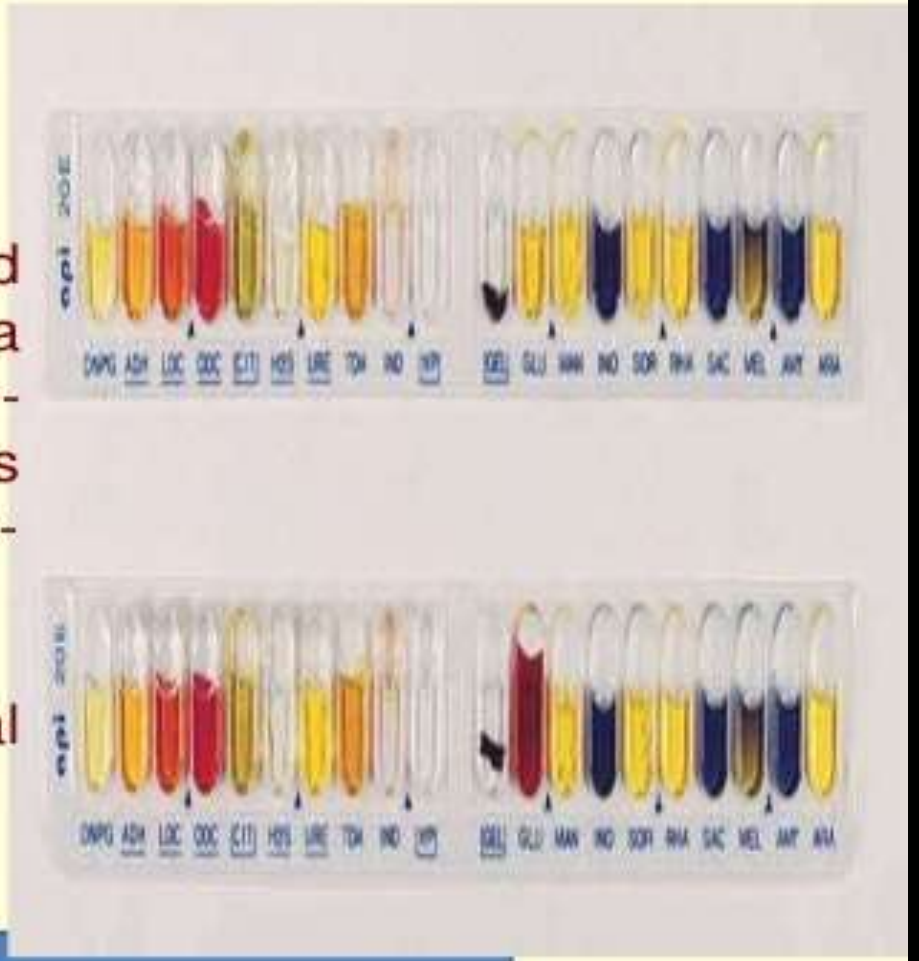
The kits usually contain 10 to 20 tests. The test results are converted to numerical biochemical profiles that are identified by using a codebook or a computer

.

API Strips - Rapid Tests

Commercial miniaturized biochemical test panels - Cover a significant number of clinically-important groups of bacteria, as well as food- and water-associated microorganisms.

The earliest, is the Analytical Profile Index (API) panel.



HOME SCIENCE

FOOD MICROBES

Automated instruments can be used to identify most Gram-negative fermenters, nonfermenters, and Gram-positive bacteria, but not for anaerobes.

Antimicrobial susceptibility testing can be performed for some microorganisms with this equipment, with results expressed as approximate minimum inhibitory drug concentrations. **Both tasks take 4 to 24**

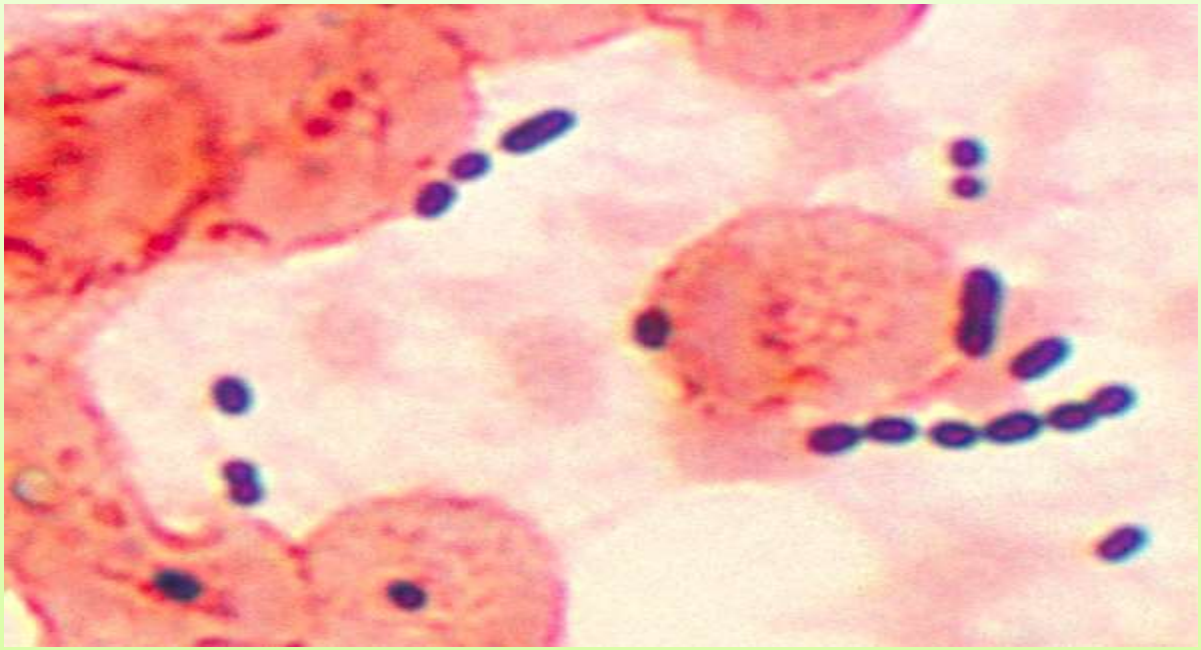
❖ Rapid Direct Testing

RDT for infectious diseases based largely on;

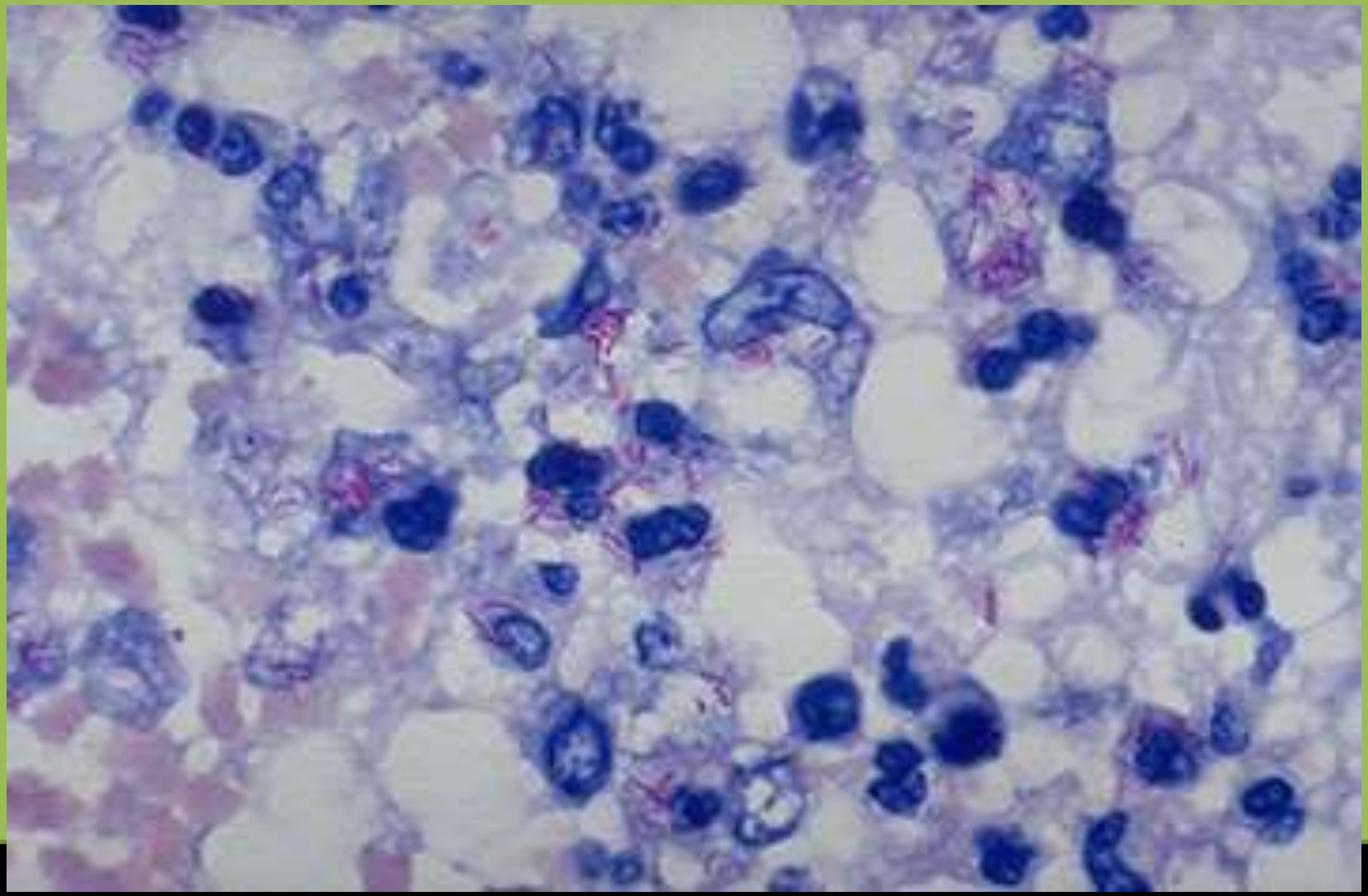
- Rapid microscopy.
- Or immunochromatography.

Rapid microscopy

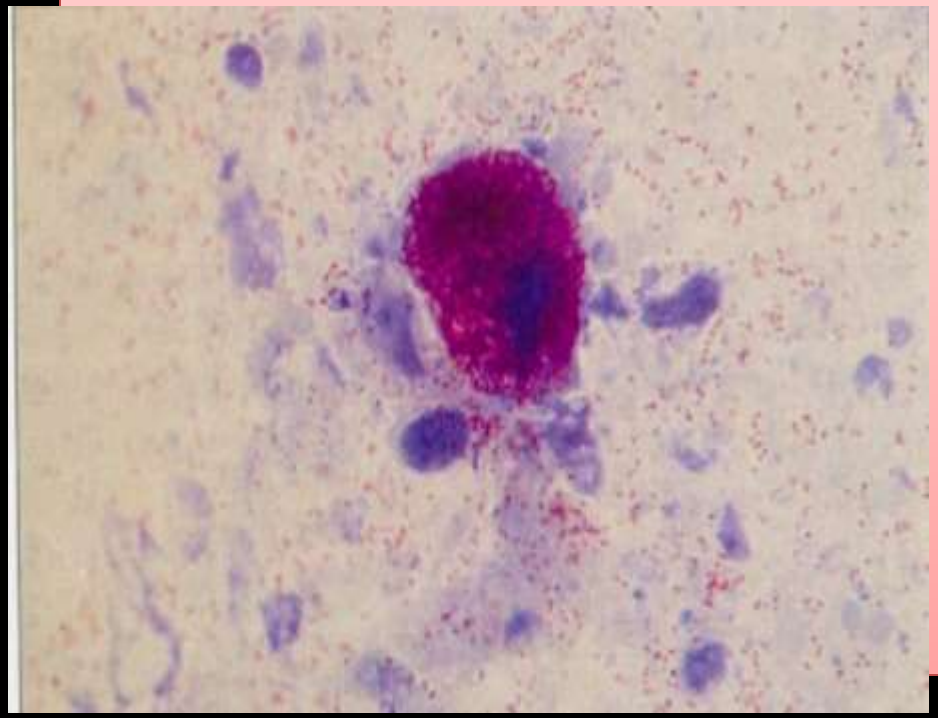
A Gram stain can confirm within minutes the presence of gram-positive diplococcic (eg, *Streptococcus pneumoniae)*

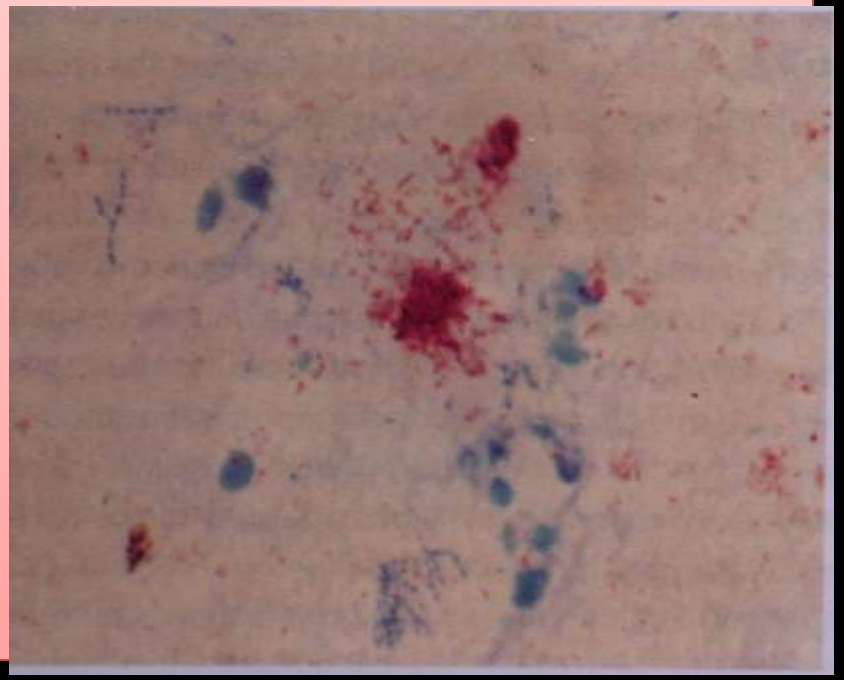


Acid fast bacilli in the sputum of a tuberculous patient

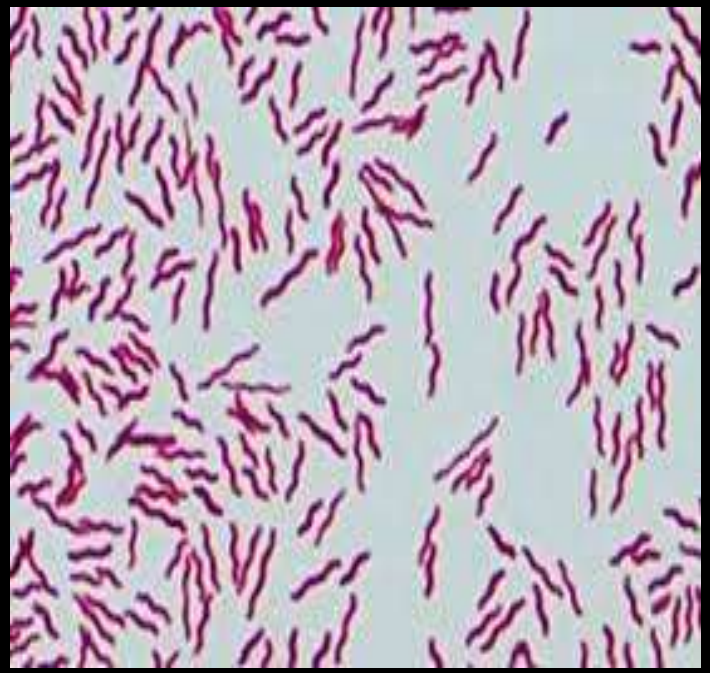


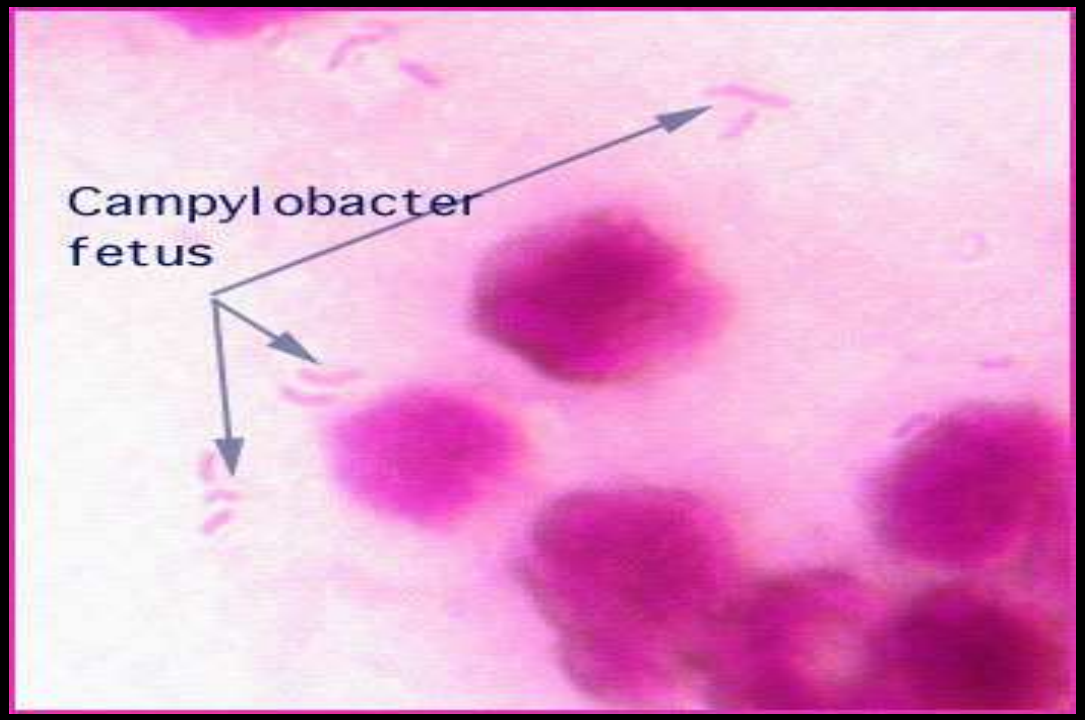
Contagious abortion (Bucellosis) may be diagnosed in few minutes after examining a direct cotyledonary, vaginal or fetal stomach content smear stained with weak acid fast stain (Stamp stain).



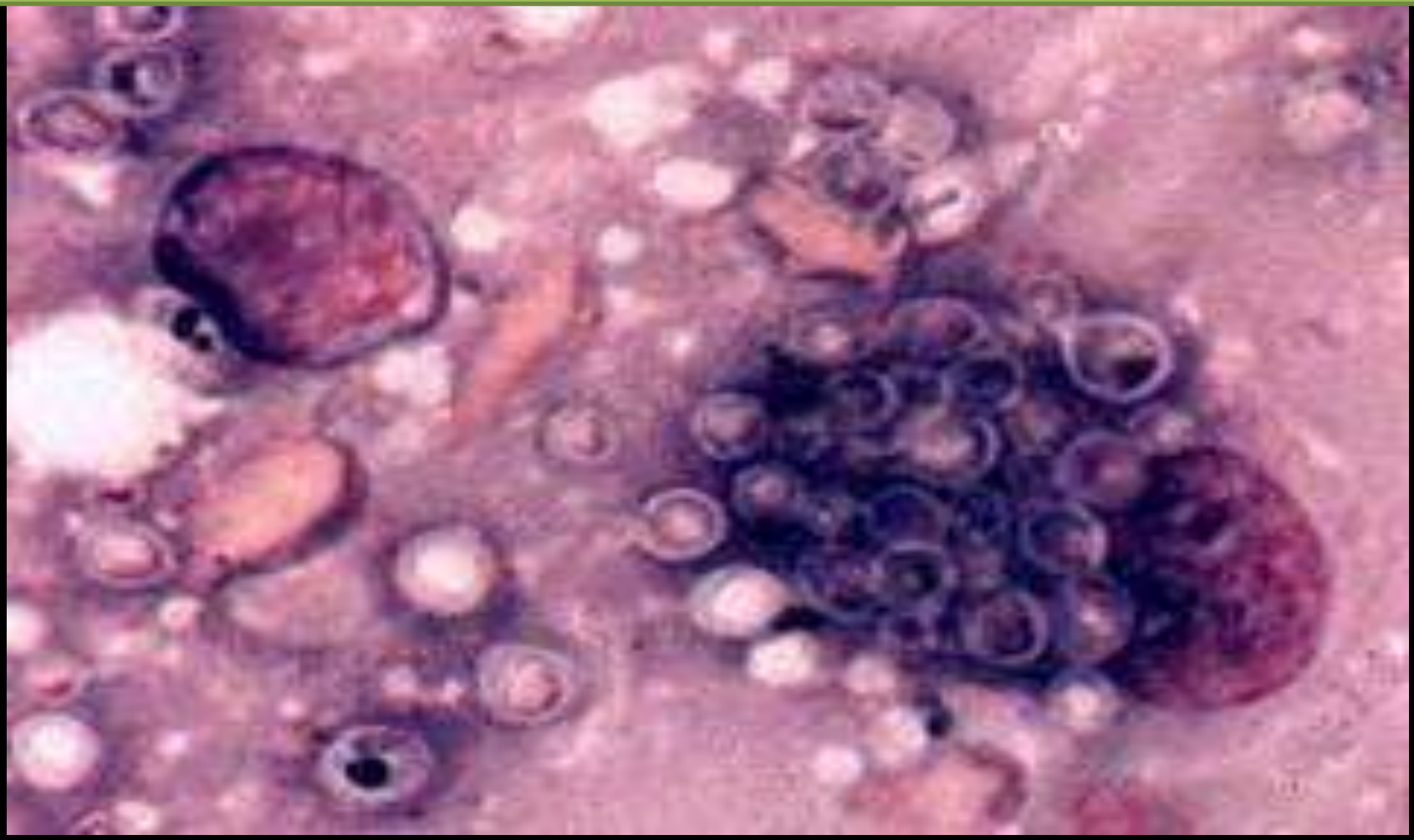


Campylobacter fetus in stomach contents of fetus and from culture

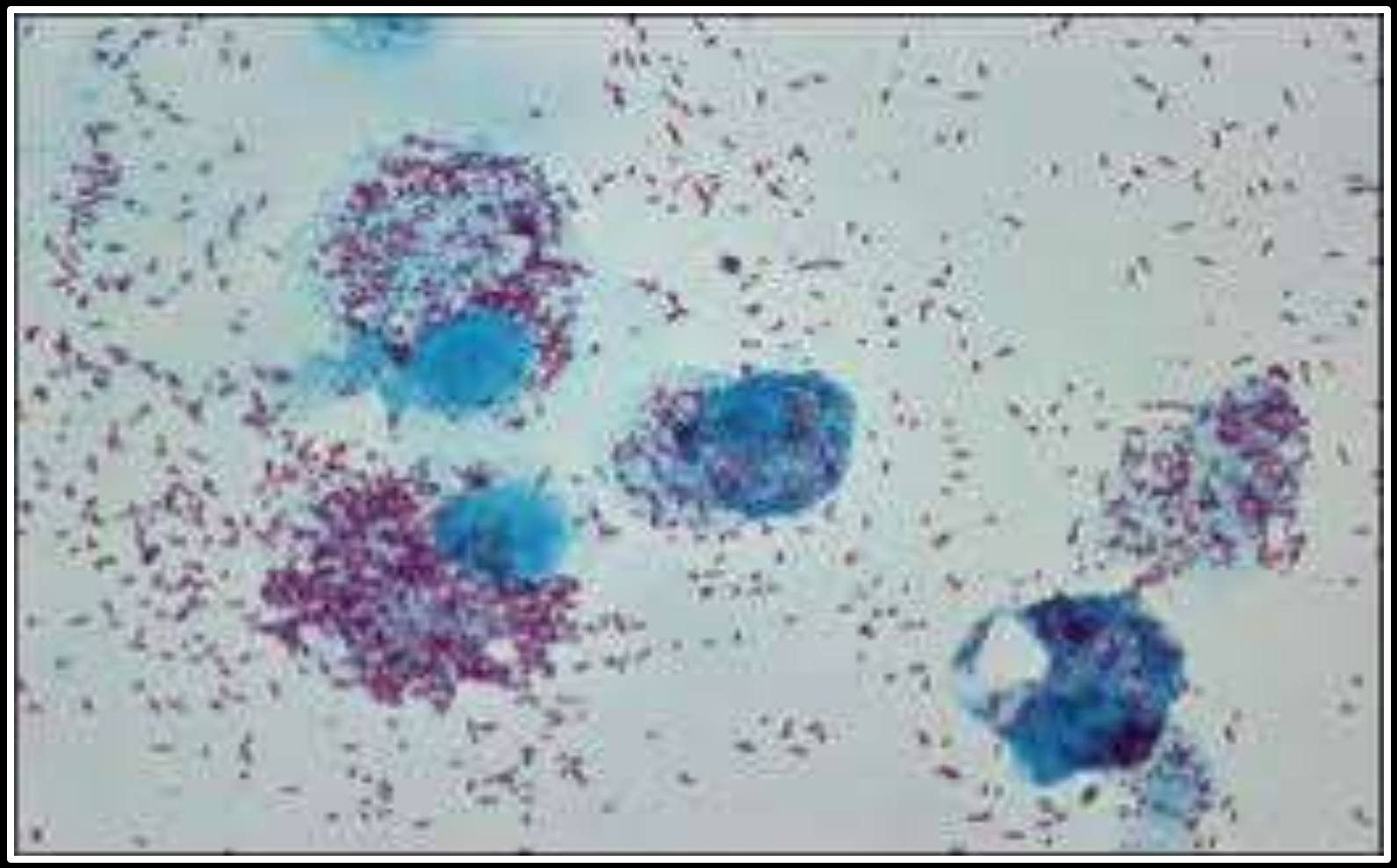




Epizootic lymphangitis, direct Gram- stained pus smear
Histoplasma capsulatum var. Farciminosum



Chlamydophila abortus- direct cotyledonary smear



Capsulated Bacillus anthracis
Direct blood film

Immunological tests

Detection of antibodies

Detection of antigens

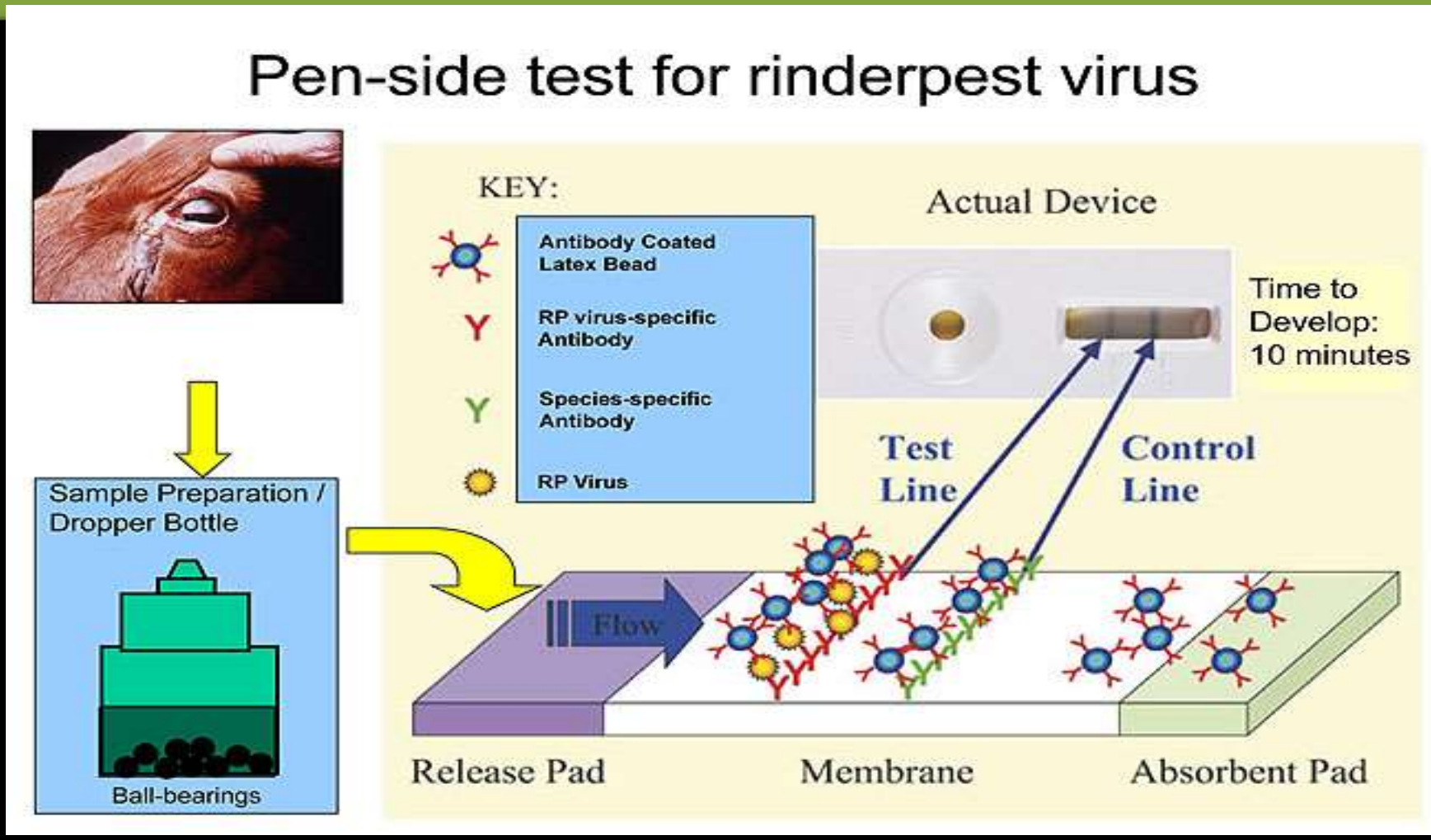
- Some antigens of different organisms are similar enough on a molecular basis …….the production of antibodies will be cross-reacting …….false positive reactions in any serological test.
- Certain types of antibody are more likely to cause false positive reactions than others.

Advances in biotechnology

- Have had and will continue to have great impact on diagnostic techniques.
- The use of highly specific monoclonal antibodies.
- Highly defined recombinant antigens

Improved the diagnostic performance of many serological tests

Immunochromatography
Pen-side (on farm) strip test Rinderpest



- a pen-side (on farm) strip test based on the same technology as used for pregnancy tests
- . Eye swabs shaken in a small volume of liquid, some of which applied to the lower window, which already contained dried reagents.

- These, and any virus particles present in the sample, then travelled upwards by wicking\capillary action.
- **If virus was present then a coloured line appeared, within minutes, in the middle window, the top window being a control.**



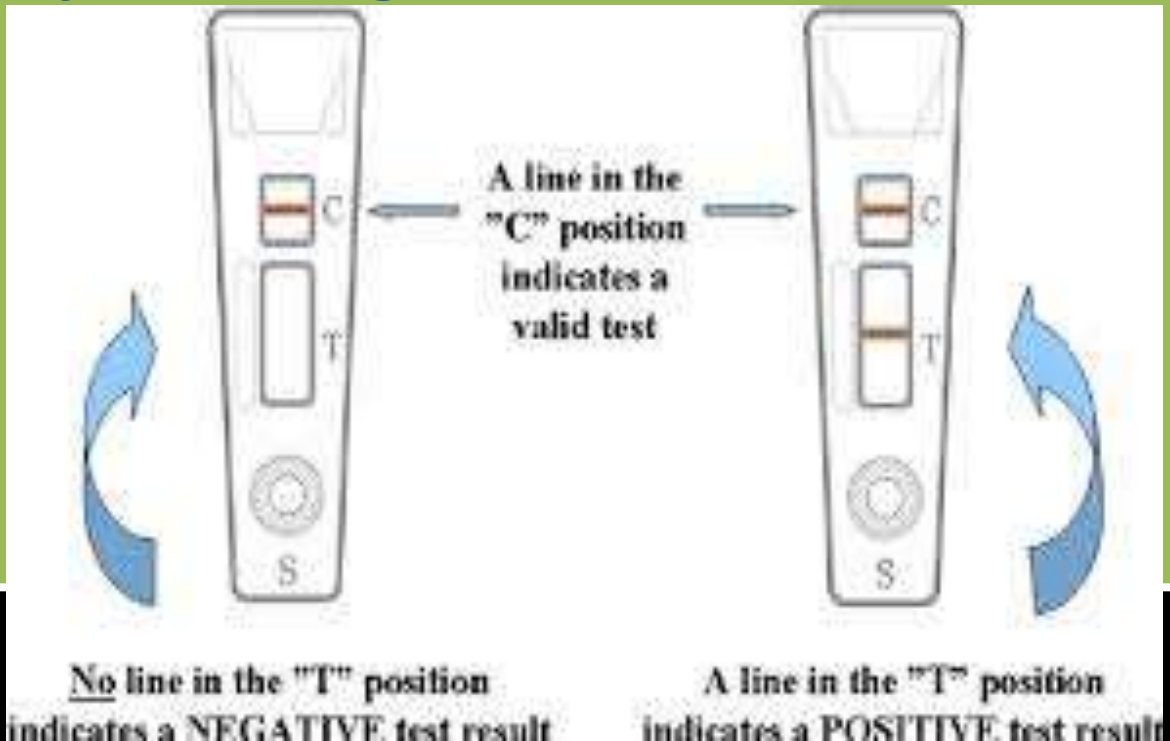
- When tested in the field ;the device is easy to use.
- Moreover, it was more sensitive than the immune-capture ELISA.
- Used to investigate potential outbreaks in developing countries.
- It revealed pockets of infection, contributing to the eradication of the virus.

- Foot-and-mouth disease (FMD) is the most devastating disease of farm animals in the world. It can destroy food supplies and farmers' livelihoods almost overnight because of the wide number of cloven-hoofed animals that are affected,

FMD

- Excretion of FMD virus can begin up to **14 days BEFORE clinical disease becomes apparent.**
- FMD virus (FMDV) can persist in the oropharynx for years following the resolution of acute infection.

lateral flow device (LFD)for the detection of foot-and-mouth disease virus in clinical samples using a monoclonal antibody



the LFD may be used next to the animal in the pen-side

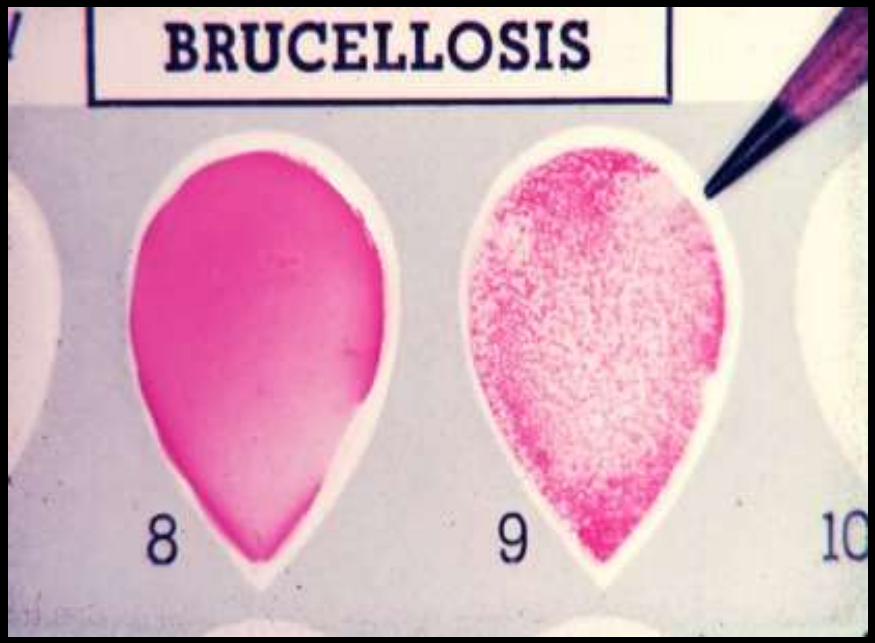
- **No reactions were detected with the viruses of diseases and that produce clinically indistinguishable syndromes in pigs and cattle.**
- The test procedure was simple and rapid, and typically provided a result within **1–10min** of sample addition.

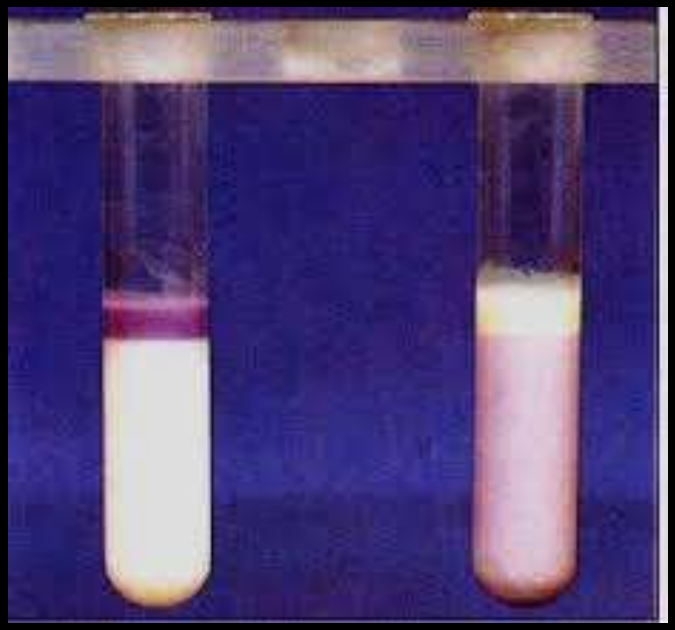
Classical serological tests

- Rely on antibody- dependent phenomena occurring as a consequence of the binding of antibody to a specific

  antigen.
- Precipitation of soluble antigens,
- Agglutination of bacteria or red blood cells,
- Lyses of red blood cells,
- Neutralization of virus infectivity.

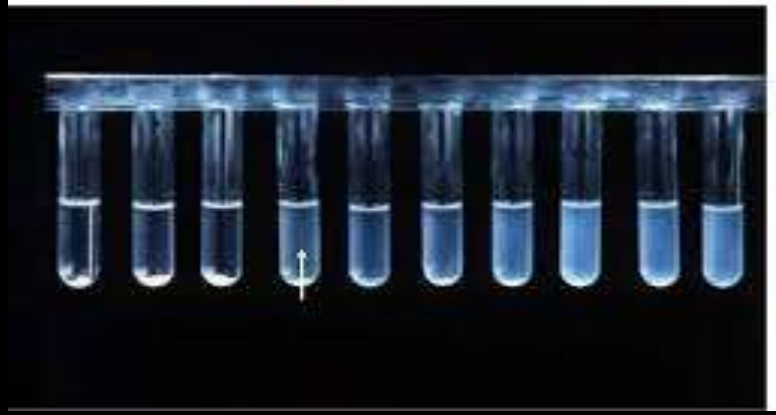
primary binding assays





Slide Agglutination Test

Tube Agglutination Test



primary binding assays

Through cloning and expression of specific proteins produced by a pathogen.

use of expression vectors and peptide synthesis have made possible the production of vector vaccines

- made it possible to produce specific proteins or peptides that serve as target antigens or positive control reagents in existing and newly-developed immunoassays.
- The use of these improved antigens can increase the specificity or sensitivity of immunoassays by providing a more defined target for binding antibody when using a more homogenous antigen.

- The widespread use of vaccines for certain diseases complicates serological diagnosis
- vaccine antigens induce antibody production which cannot readily be distinguished from antibodies produced due to infection.

This can be accomplished by either:

*Expressing a single immunogen in a vectored vaccine.

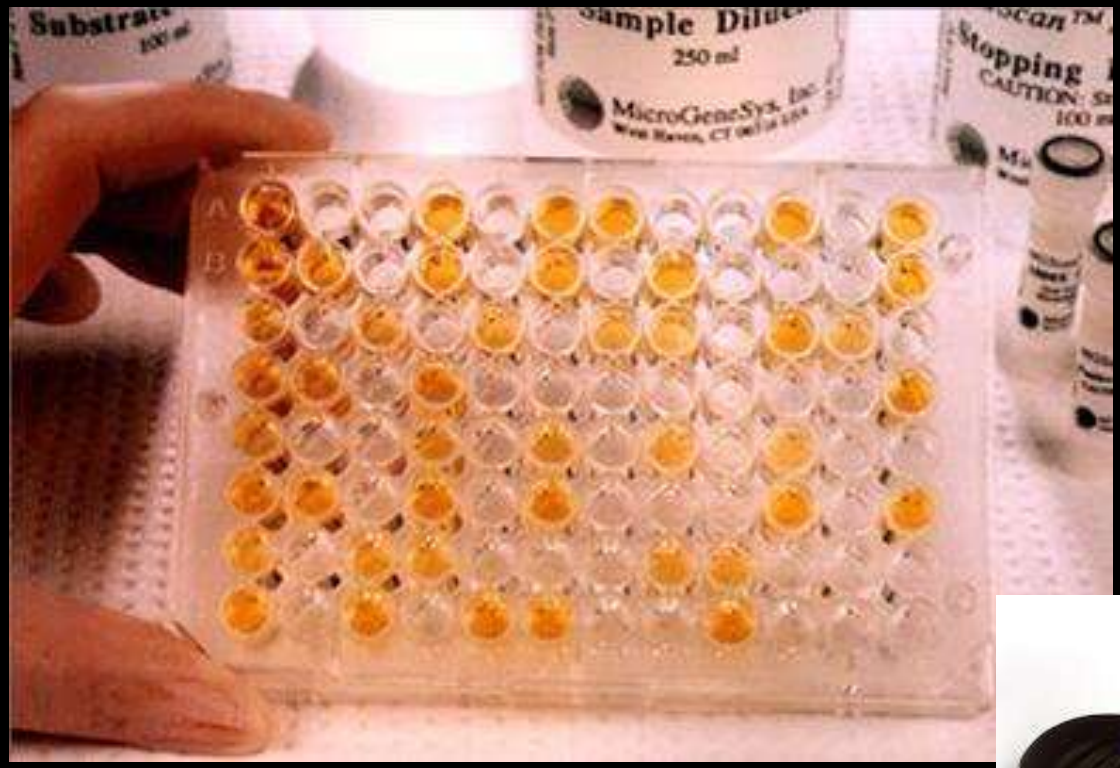
*Deleting the expression of a single immunogen in a vaccine.

Followed by the development of a complementary serological assay.

Animals vaccinated with a gene-deleted vaccine do not make antibodies specific to the unexpressed protein,

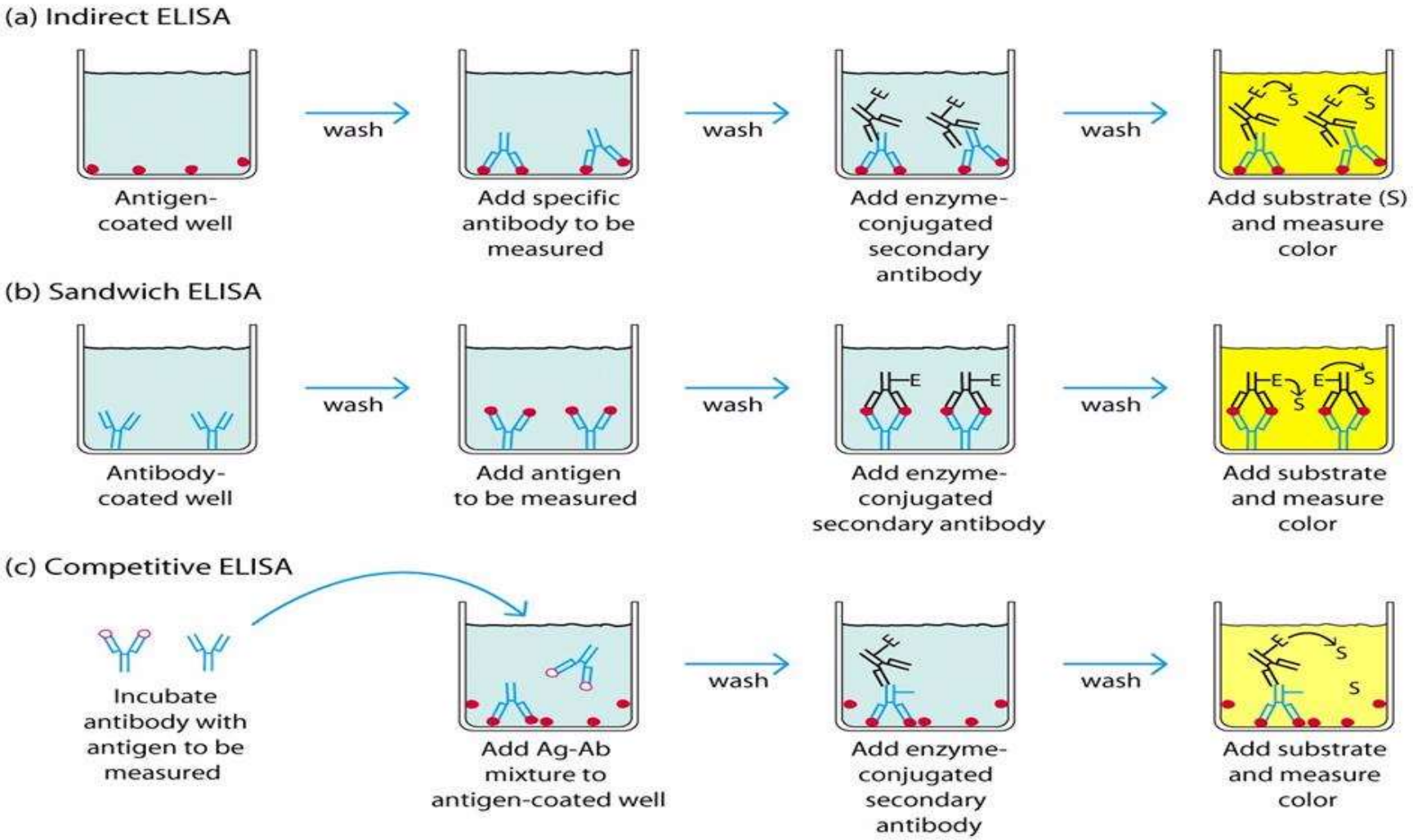
It make antibodies to the other immunogens produced by the pathogen, resulting in a protective immune response.

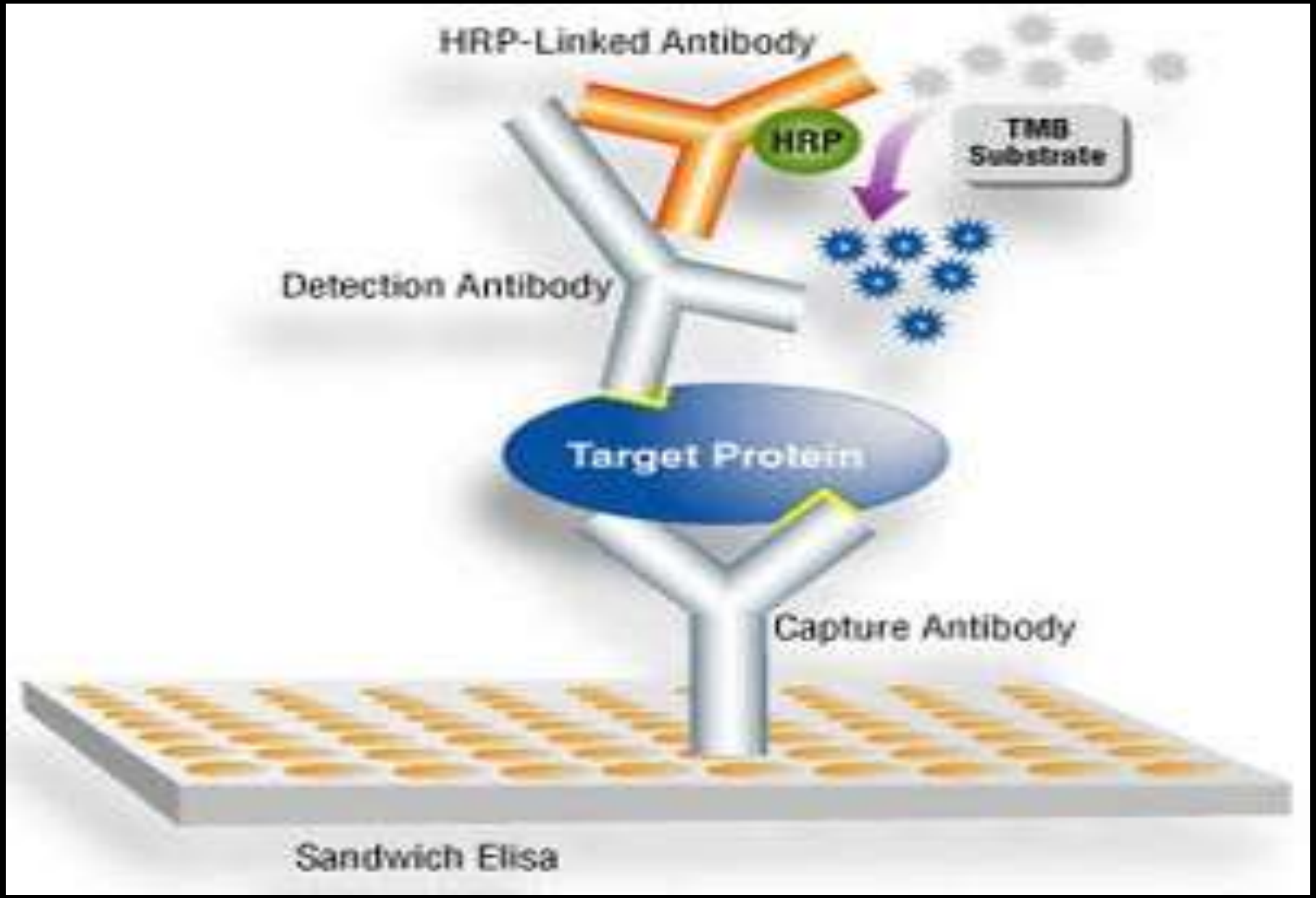
Serologic method



ELISA

Імуноферментний метод




HRP-Linked Antibody
HRP
TMB Substrate
Detection Antibody
Target Protein
Capture Antibody
Sandwich Elisa

Aujeszky's disease (pseudorabies)

Aujeszky's disease (pseudorabies) eradication programmes

Vaccines were developed with a specific gene deletion

which prevented the expression of a specific protein.

This approach enabled Aujeszky's disease programmes to achieve eradication more rapidly;

by:

Establishing a vaccinated population of animals in which infection with field strains of virus could be detected.

A complementary ELISA

Using the specific gene-deleted protein from the vaccine could then detect antibodies from infected vaccinated and non-vaccinated animals with the field strain, but not uninfected vaccinated animals.

FMD Infected and FMD vaccinated animals

Recently-developed ELISAs are able to distinguish.

- Virus replication during infection results in the production of several non-structural (NS) proteins, some of which are immunogenic.
- while inactivated vaccines induce antibodies almost exclusively to structural proteins.

Definitions

A **nonstructural protein** is a protein encoded by a virus but it is not part of the viral particle.

A **viral structural protein** is a viral protein that is a structural component of the mature virus

Detection of infectious antigen

Immuno-histochemical meth

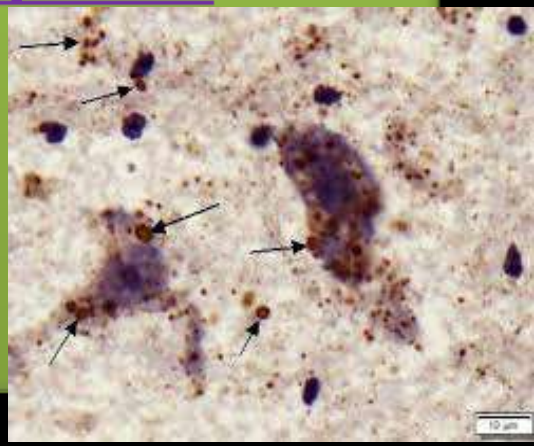
Direct Rapid Immunohistochemical Test:

it can be performed within two hours without the need of fluorescent microscope.

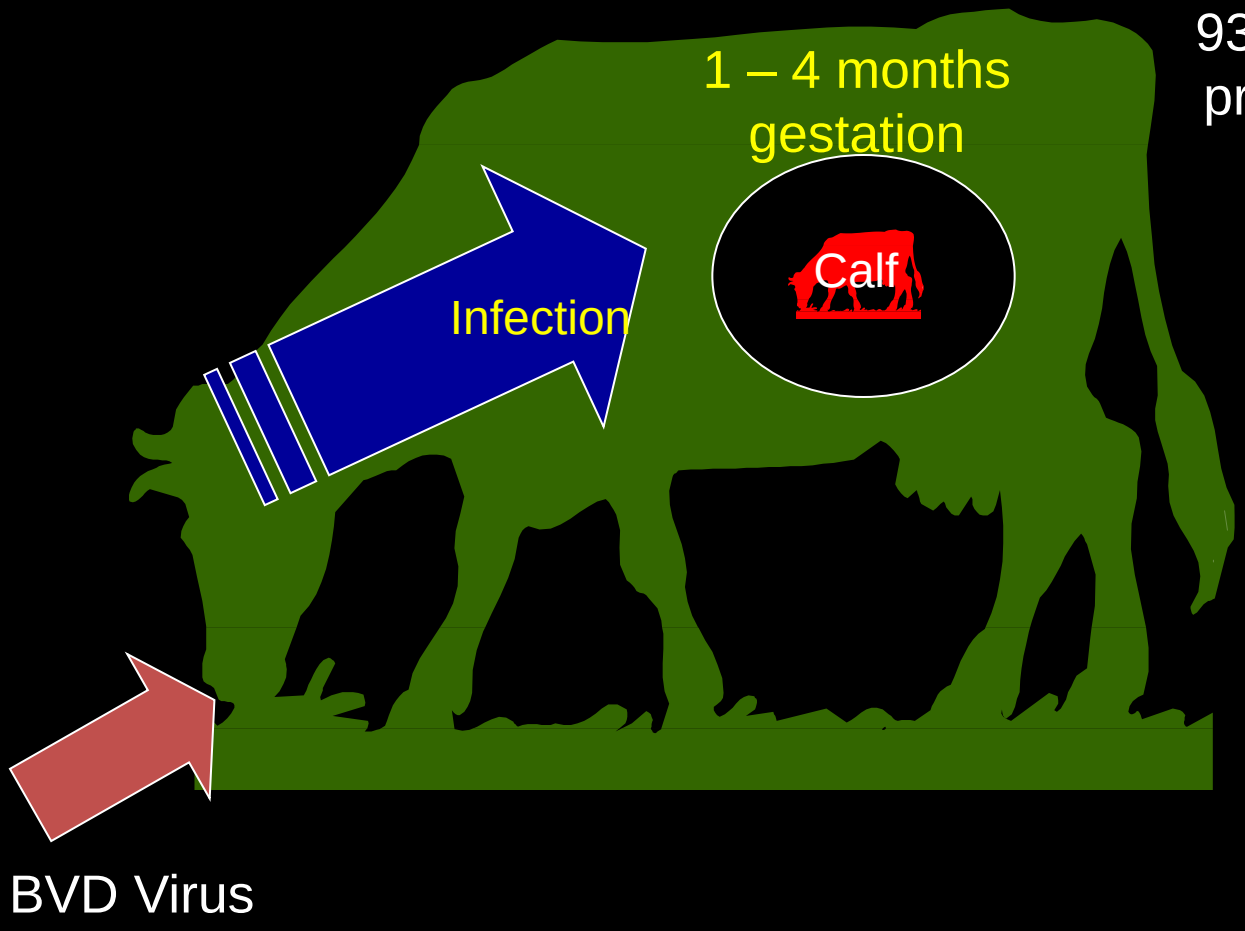
By detecting rabies N protein in suspected brain smears fixed in buffered formalin **using a cocktail of highly concentrated and purified biotinylated monoclonal antibody to N protein** followed by addition of streptavidin peroxidase and substrate colouring reagent.

Rabies

The N antigens, if present, appeared as brownish red clusters within the neuron, along the axons and scattered all over the brain smears. **It has the advantage of applicability under field conditions as expensive fluorescence microscope is not required**



Bovine viral diarrhea (BVD)
Persistently Infected Carriers (PI's)



93% of all PI's produced this way

PI's produce PI's 100% of the time

PI BVD

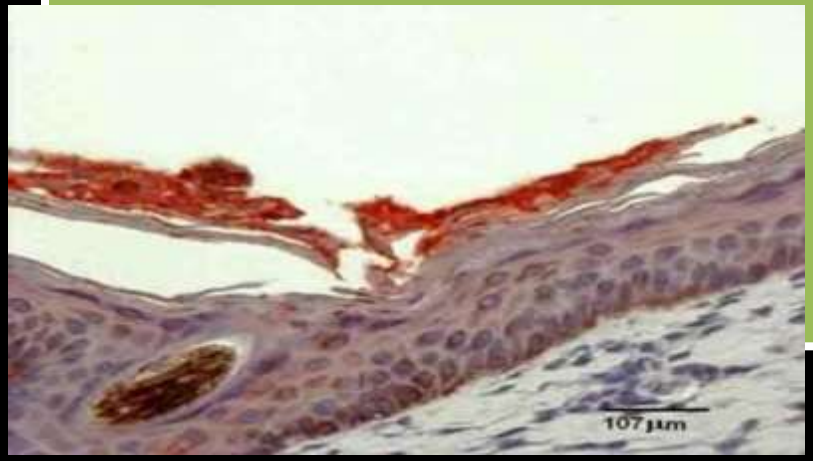
- Colostrum-derived antibodies neutralize virus in serum for up to 4 months, diagnosis of persistently BVDV-infected neonatal calves difficult and expensive.

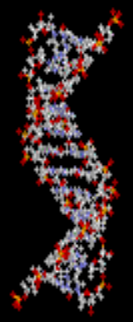
Early Calve in utero infection...

..No antibodies

1- to 4-week-old dairy calves were screened for BVDV using Formalin-fixed skin biopsy samples stained for BVDV antigen by Immunohistochemistry (IHC),

Skin biopsy represents an effective method for identifying animals PI with BVDV

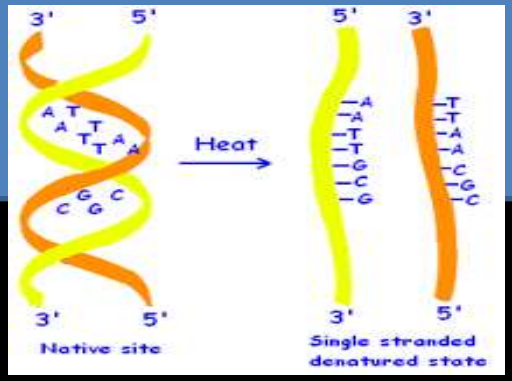




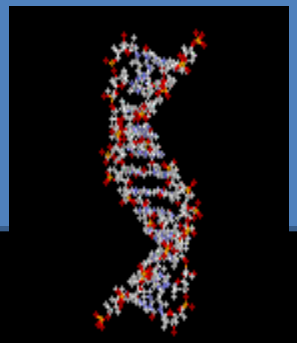
Polymerase Chain Reaction

PCR

Technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.



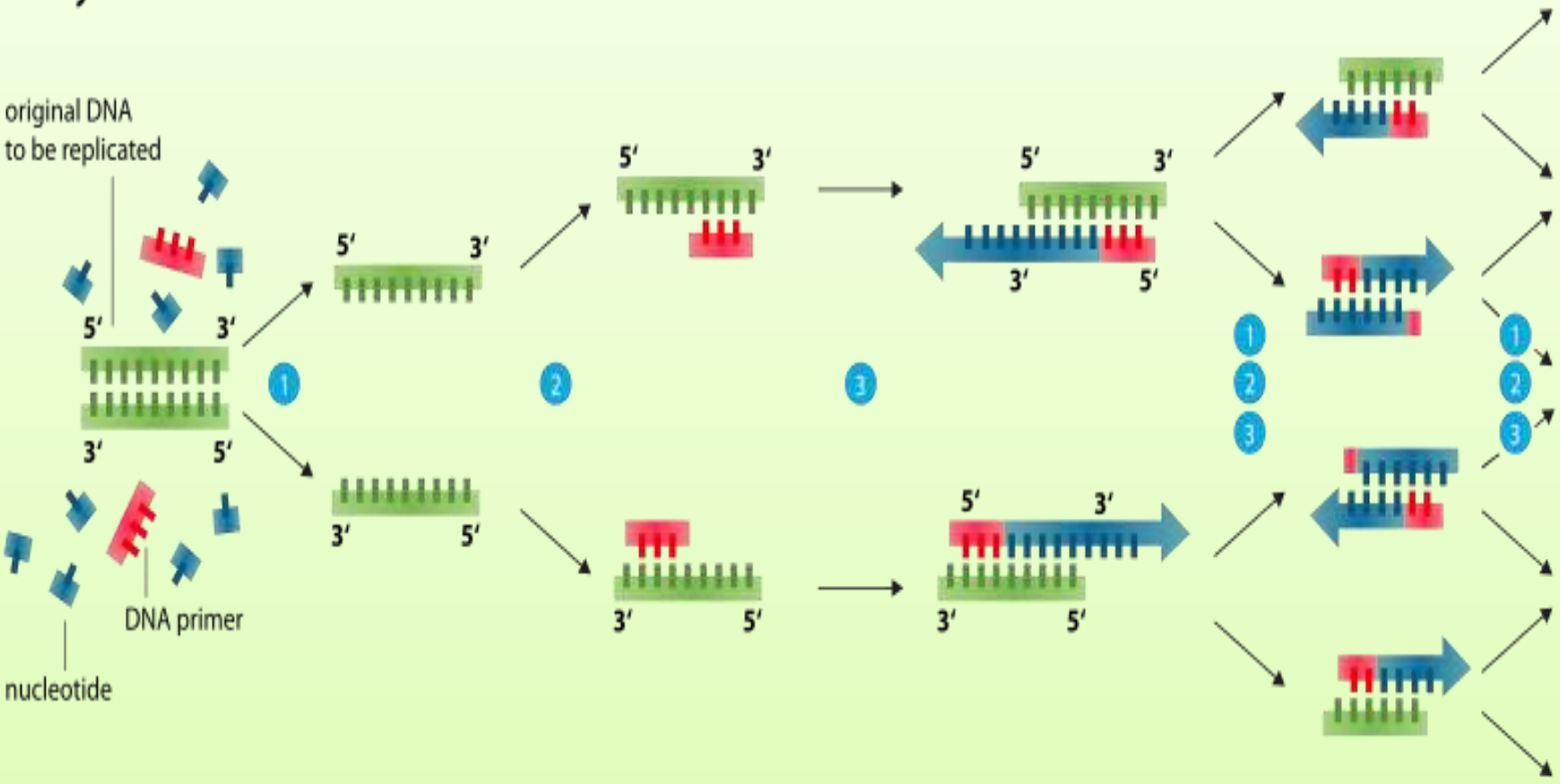
PCR



- Extraction and purification of nucleic acid (NA).
- Amplification or making copies of NA of interest (target).
- DNA sequences in an exponential fashion by in vitro DNA synthesis.
- Detection of the amplified target using RT-PCR, or end product detection.

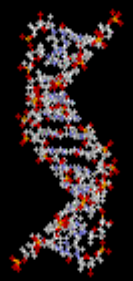
The Reaction

Polymerase chain reaction - PCR



1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C

Real time PCR (RT-PCR)



The most recent development in the field of diagnosis by nucleic acid detection is the use of thermal cyclers capable of measuring **fluorogenic PCR amplification in real-time.**

The fluorogenic RT-PCR provides relatively fast result, enables a quantitative assessment to be made of virus amount, and can handle more samples and/or replicates of samples in a single assay than the conventional RT-PCR

RT-PCR

- Rt-PCR made it possible to test large numbers of samples in a matter of hours during disease outbreaks.
- In addition, real-time PCR has been adapted for use in the field through the use of portable thermocyclers and lyophilized reagents.

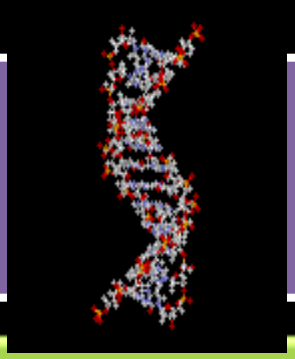
Field PCR FMD

- FMDV molecular diagnostics Multiplex RT-PCR Very sensitive, simple, takes 4h, Ability to serotype.
- Non-specialist user – Nucleic acid extraction – PCR set-up – Analysis modules.
- Battery operated.
- Easily decontaminated.

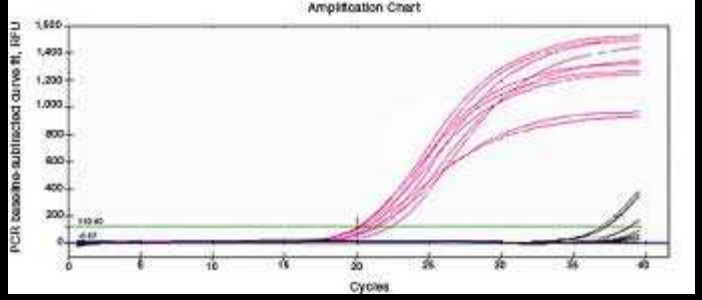
FMD

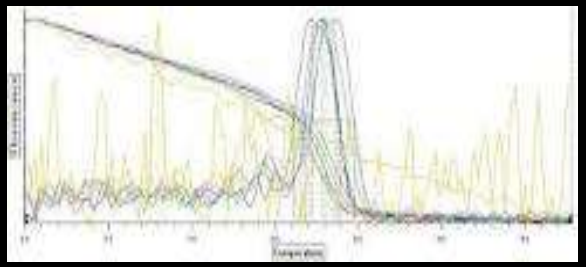
- Measurement of viral shedding by taking nasopharyngeal swabs and applying Real-Time RT-PCR

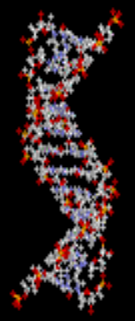
RT-PCR



- Detection of positive samples is dependent on the amount of fluorescence released during amplification.
- **These fluorescence readings are plotted by computer software and results can be transmitted electronically, eliminating the need for post-PCR reaction analysis by electrophoresis**







- In the past, the amplified PCR products were revealed by electrophoresis in an agarose gel (gel-based PCR). By real-time PCR, where the PCR product is monitored in each cycle of amplification (ie, in real-time) by the use of a double-stranded DNA fluorescent dye or a fluorescein-labeled probe.
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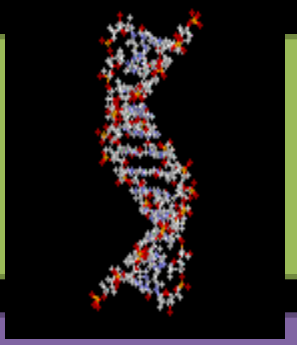
Nested PCR



- Refers to the application of a second set of primers targeting a shorter area on the first-stage amplified product (DNA).
- Using this approach increases the sensitivity of the PCR and generates two amplified products for confirmation purposes

Multiplex-PCR (mPCR)

- **The development of multicolour real-time PCR cyclers and "ready-to-use" commercial multiplex real-time PCR kits made it possible**
- **Combine several assays within a single tube.**
- **Reduced sample requirement,**
- **Ability to combine assays with an internal control system.**

Multiplex PCR (mPCR)

Differentiating FMDV serotypes.

Differential diagnosis with other vesicular diseases such as Vesicular' Stomatitis.

Mastitis

In more than 30% of milk samples from clinical and subclinical bovine mastitis, bacteria fail to grow.

The no growth samples represent a problem.

Investigation of the bacteriological etiology of such samples, using a real-time PCR-based commercial reagent kit.

PCR & Diagnosis of mastitis

Bacterial DNA is extracted from milk samples.

Mixed with “primers” are templates of known nucleotide sequences from particular bacterial speci(strains).

Mastitis

- The assay targets the DNA of the 11 most common bacterial species or groups in mastitis.
- The staphylococcal gene responsible for penicillin Resistance;
- Can identify and quantify bacterial cells even if dead or growth-inhibited.

THANK YOU FOR YOUR TIME AND ATTENTION