Diagnosis Techniques Diseases in Camel Dromedary

DR. MEHDI EL HARRAK

Laboratory Orientation and Safety

- Prevention of physical injuries and laboratory acquired infections with zoonotic disease agents
- Laboratory safety is every employee's responsibility!

What is a Zoonotic Disease?

- "Diseases and infections naturally transmitted between vertebrate animals and man, with or without an arthropod intermediate" WHO
- Of the >1,700 known pathogens affecting humans, 60% are zoonotic
- Of the 156 pathogens associated with emerging diseases, 75% are zoonotic
- 80% of potential bioterrorism agents are of animal origin

Most of significant disease of camel are potentially zoonotic

Biosafety Levels (BSL) Defined

- BSL-1: Level to handle a microorganism **not known to cause disease in humans**, with minimal community risk
- BSL-2: Level to handle a microorganism that **causes human disease**, with minimal community risk
- BSL-3: Level to handle a microorganism that **causes serious (or potentially lethal) human disease**
- BSL-4: Level to handle a microorganism that **causes life threatening disease** in humans

Containment/Barriers

- Equipment : "primary barriers"

Biological safety cabinets (BSCs) – Class II Provides personnel, environment, and specimen protection (Personal Protective Equipment PPE)

Aerosol-resistant centrifuge cup holders

- Facilities : "secondary barriers"

Building design to control traffic

Air flow/HEPA filtration

Sewage/waste treatment

Standard Laboratory Safety Practices

- Use mechanical pipetting devices
- Wear disposable gloves/wash hands frequently
- Avoid touching eyes, face
- Decontaminate work surfaces after each activity
- Clean and disinfect spills and splashes promptly
- Restrict or limit access to laboratory
- Prohibit eating, drinking and smoking
- Proper disposal of waste materials

Diagnosis of diseases of camel

- **Presumptive diagnosis**

  Clinical signs/lesions
  Epidemiological context
  Serologic diagnosis

- **Definitive diagnosis**

  Molecular detection
  Isolation and characterization of the pathogen w/subtyping/pathotyping

Source of Samples

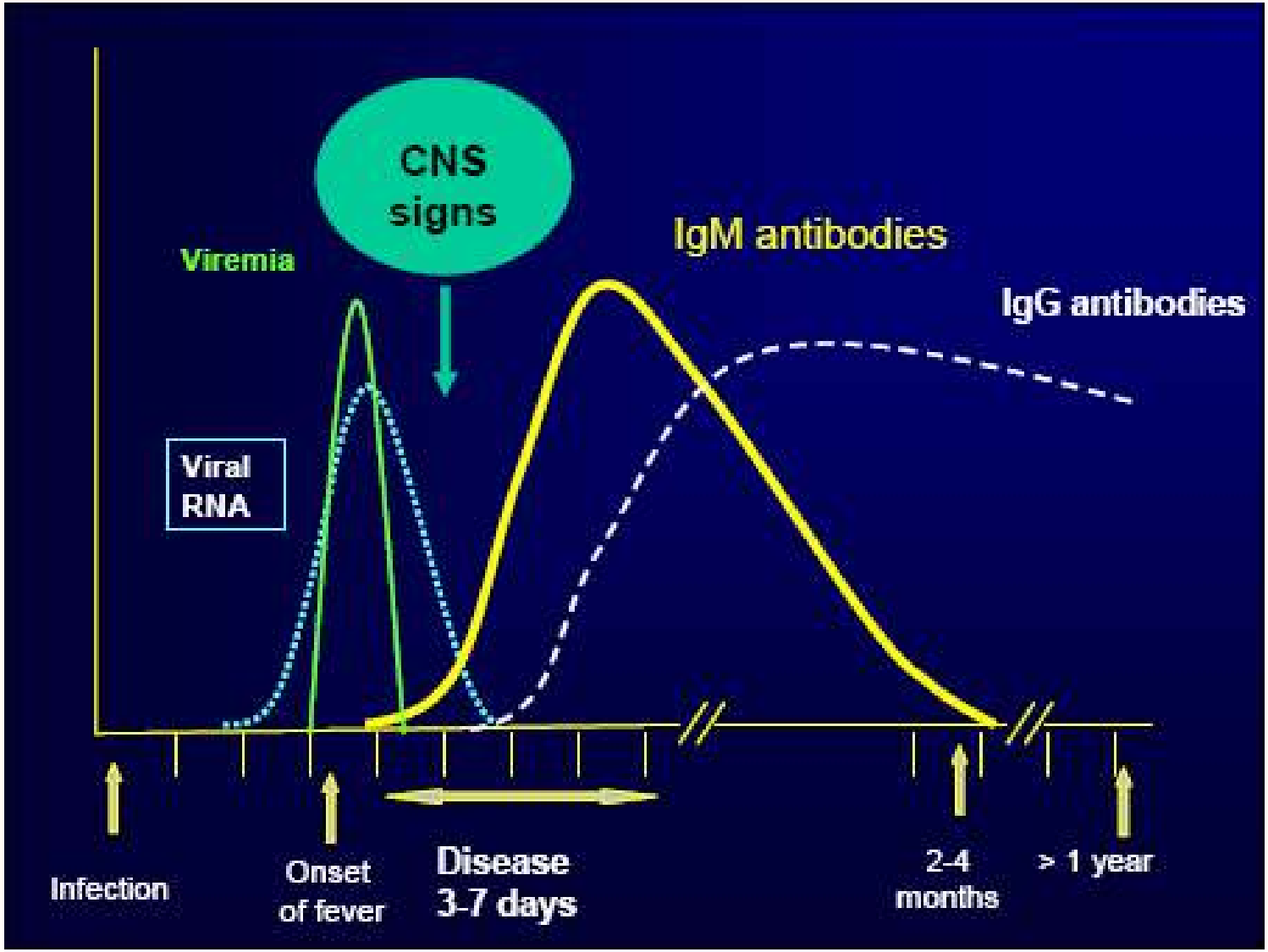
- **Passive surveillance**

  Investigations of clinical cases

- **Active surveillance (random, organized)**

  Live animal markets
  Processing plants– slaughter
  Export testing
  Pre slaughter/movement

Direct and indirect diagnosis



Serology Tests

- Agar gel immunodiffusion (AGID) test IgM, (IgG)
- Enzyme-linked immunosorbent assay (ELISA) IgG
- Hemaggltination-inhibition test
- Complement fixation test
- Immuno fluorescence
- Seroneutralization test

Serology: Types of Samples

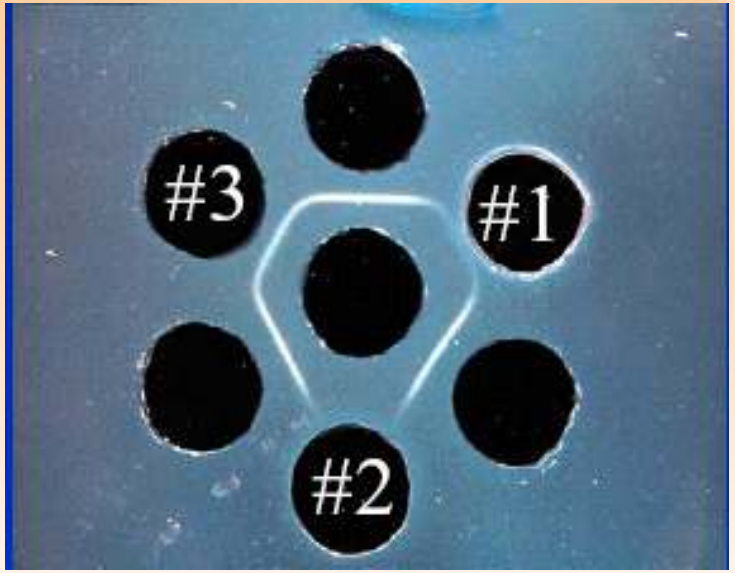
CAUTION!

Serology tests should be used to determine the immune status of a flock, not an individual camel

AGID Test

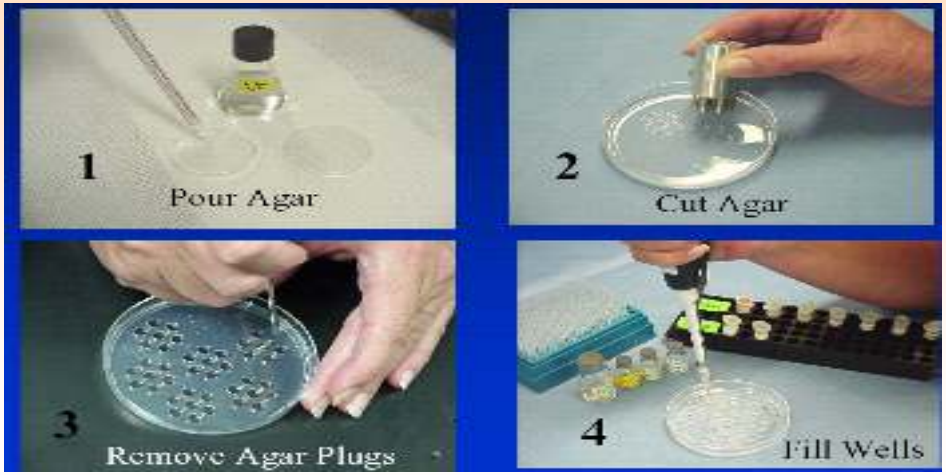
- **Advantages:**
  - Gold Standard (screening)
  - Detects antibody to all virus serotypes
  - Easy, inexpensive, requires few reagents/equip

- **Disadvantages:**
  - Semi quantitative
  - Moderate sensitivity
  - Subjective interpretation
  - Requires 24 hr
  - Requires further testing of positives
  - Antibodies not detectable for several days



Application for camel diseases diagnosis

- Bluetongue
- Influenza
- Equine encephalitis (NW camelids)



Group Specific Test: *ELISA*

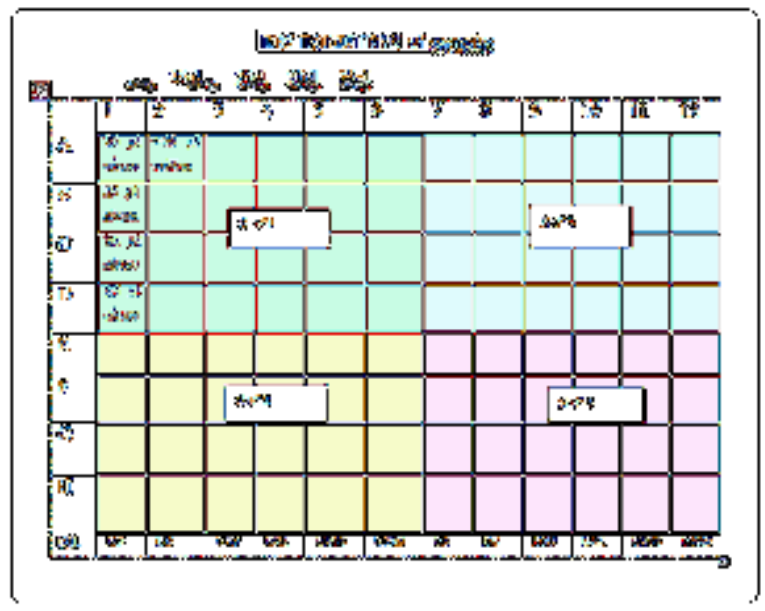
- **Advantages**
  - Commercial kits may be available
  - Rapid (same day)
  - Can be semi-automated

- **Disadvantages**
  - Requires expensive equipment
  - False positive samples
  - Positives require confirmation

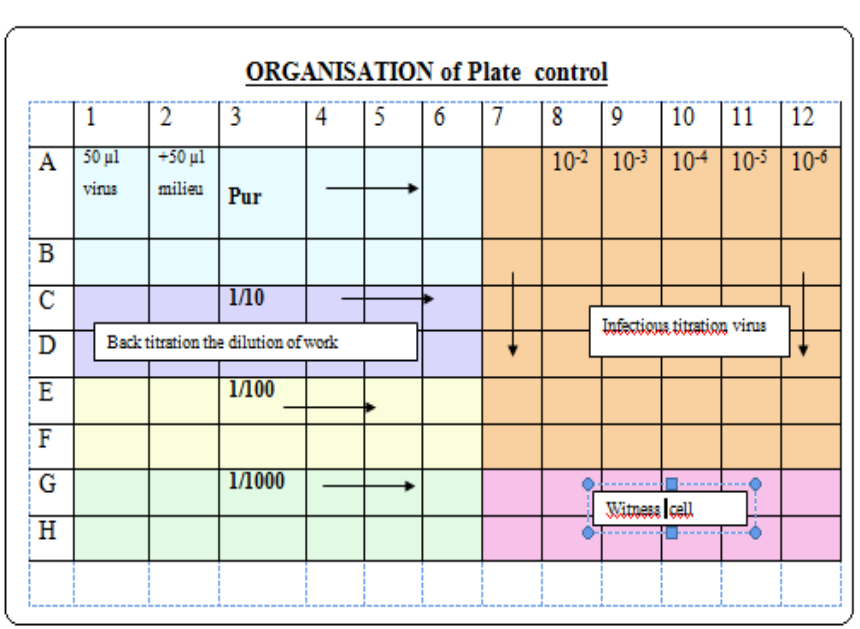
Serologic Technical Seroneutralisation

- Gold standard technique
- Performed on cell culture or embrynated eggs
- Used for the studies on the prevalence of the disease, or virus identification/serotyping.
- Neutralizing antibodies IgG persist during years after the initial infection.

Serologic Technical



Serum dilutions
Ad specific virus
Add cells
Incubation 3-7 days
Check for CPE presence or absence
Determine neutralizing titer



Application for camel diseases diagnosis

- Camelpox
- Rabies
- RVF
- BVD
- AHS, BT, EHD (serotyping)

Haemagglutination inhibition

- **Advantages**
  - Gold standard
  - Quantitative (titer)
  - Subtype/serotype specific
  - Rapid (same day)

- **Disadvantages**
  - Requires many reagents antigens/antiserums)
  - Non-specific (steric) inhibition
  - Requires pre treatment of serum to remove normal serum agglutinins (false negatives)

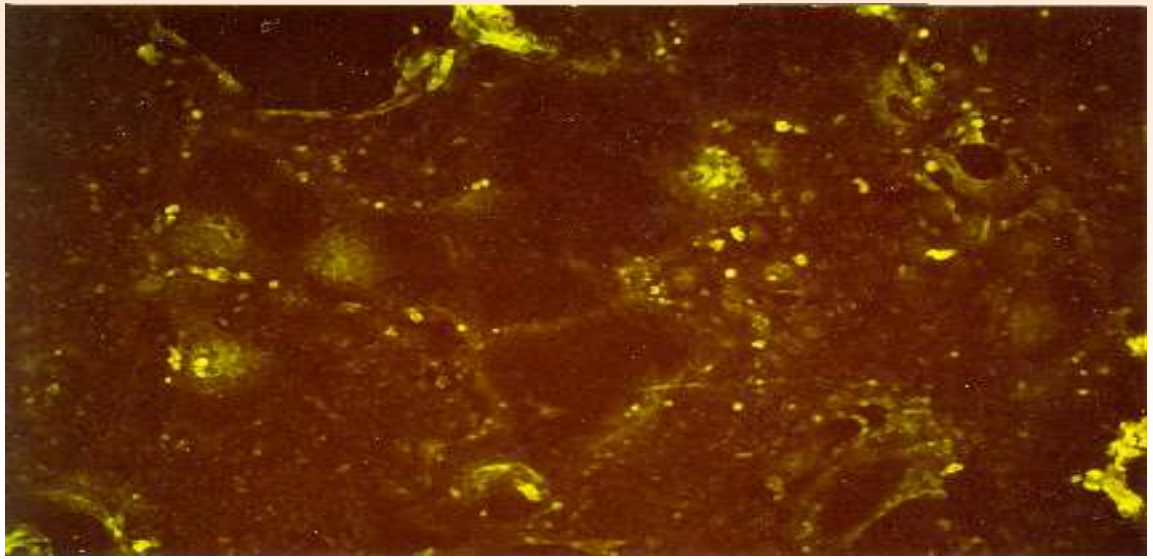
Application for camel diseases diagnosis

- Camel Pox
- Equine encephalitis (NW camelids)
- Influenza
- PPR

Serological technique

Immunofluorescence:

- Allows detection of antibodies by use of conjugated to fluoresceine antispecies Ab.
- Used in TC to identify viruses or for titration.
- Application for camel diseases diagnosis: Rabies



Diagnosis of Camel diseases
The pathogen Detection Methods

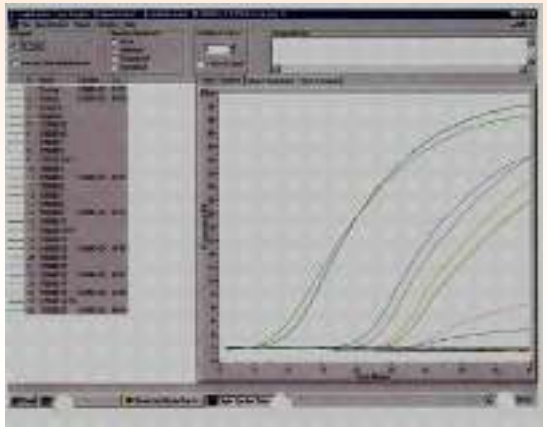
- Isolation

Required for identification characterization of the pathogen

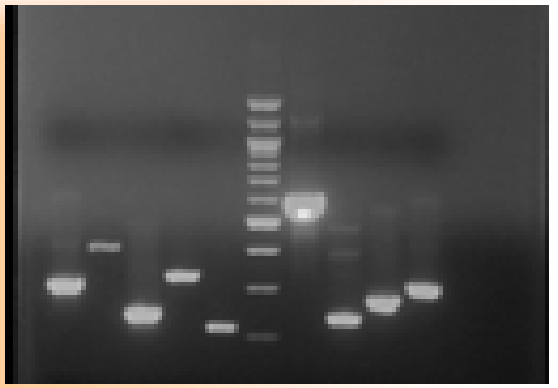
- Antigen capture Elisa
- Gel Based PCR
- Polymerase Chain Reaction (rRT-PCR)

Molecular Diagnostics
rRT-PCR

- **Samples**
  - swabs, tissue (lung, spleen, blood)
- **Advantages**
  - Rapid (2.5 hr in rt-PCR)
  - Highly Sensitive/Specific
  - Differentiates serotypes
- **Disadvantages**
  - Expensive equipment
  - Moderate per test cost ($20)
  - Special facilities required



Gel based PCR



- Perform RT-PCR in a one-step or two-step format
- Analyze DNA product on an ethidium bromide stained agarose gel
- Sensitivity can be similar to virus isolation
- Working with large amounts of amplified DNA creates a cross contamination risk!
- Numerous recommendations to reduce contamination
- Requires less expensive equipment, but greater manpower to perform test

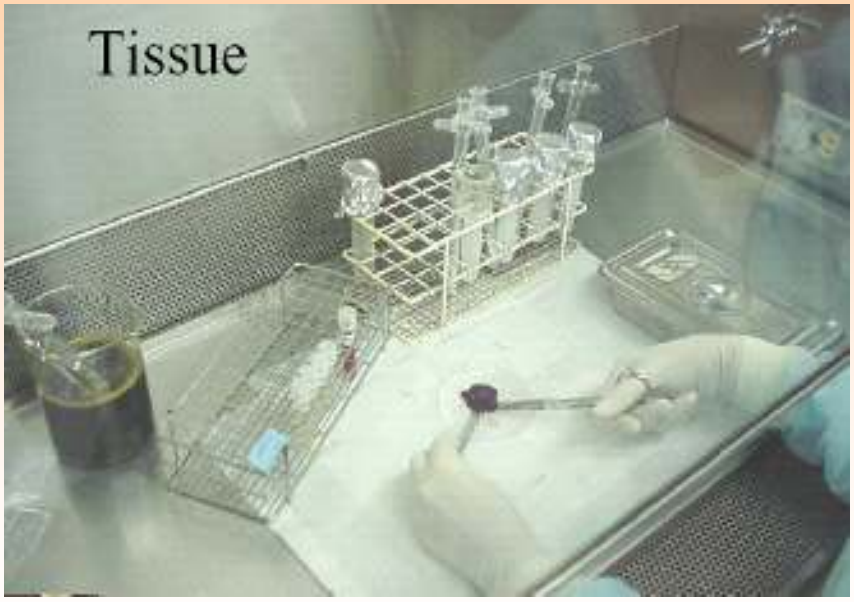
Real-time RT-PCR (rRT-PCR)

- One-Step RT-PCR test was developed for typing and subtyping of viruses using fluorescent Taqman probes
- Sensitivity similar to virus isolation
- The test doesn’t require running the PCR product on a gel and the probe confirms specificity
- The complete test, including the RNA isolation step, can be completed in less than three hours
- Requires expensive equipment, but can be done faster and cheaper than conventional virus isolation

Pathogens Isolation

- **Samples**
  - Any (tissue, swabs, blood)
- **Advantages**
  - Gold standard
  - Sensitive/Specific
- **Disadvantages**
  - Expensive and labor intensive
  - False negatives (sample mishandling)
  - Special facilities needed

Processing Specimens

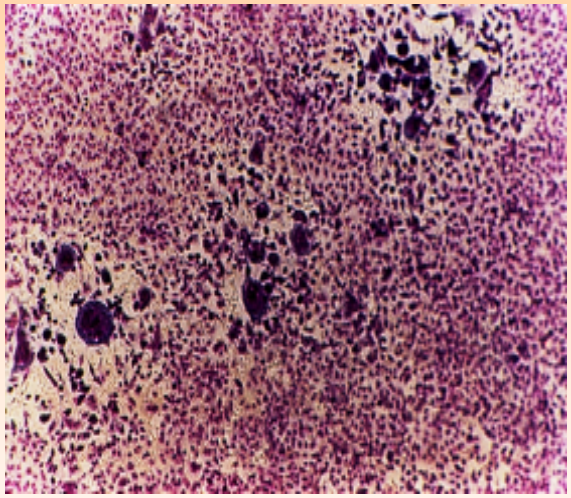


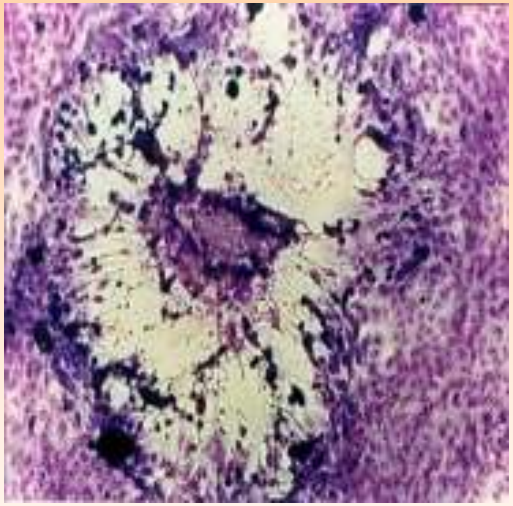
Isolation
Embryonating Eggs:

- Specific-pathogen-free (SPF) or Commercial flocks
- Inoculate between 9-11 days of incubation

Chorioallantoic sac (CAS) route intra allantoic fluid

Virus isolation on cell culture





A large variety of cell cultures including the following cell lines: Vero, MA-104 and MS monkey kidney, and, baby hamster kidney (BHK21)

VALIDATION PROCESS

- Validation is a process that determines the fitness of an assay, which has been properly developed, optimised and standardised, for an intended purpose.
- All diagnostic assays should be validated for the **species** in which they will be used.
- Validation includes estimates of the analytical and diagnostic performance characteristics of a test. An assay that has completed the first three stages of the validation pathway, including performance characterisation, can be designated as "validated for the original intended purpose(s)".
- To maintain a validated assay status, however, it is necessary to carefully monitor the assay's performance under conditions of routine use, often by tracking the behaviour of assay controls over time.

VALIDATION PURPOSES

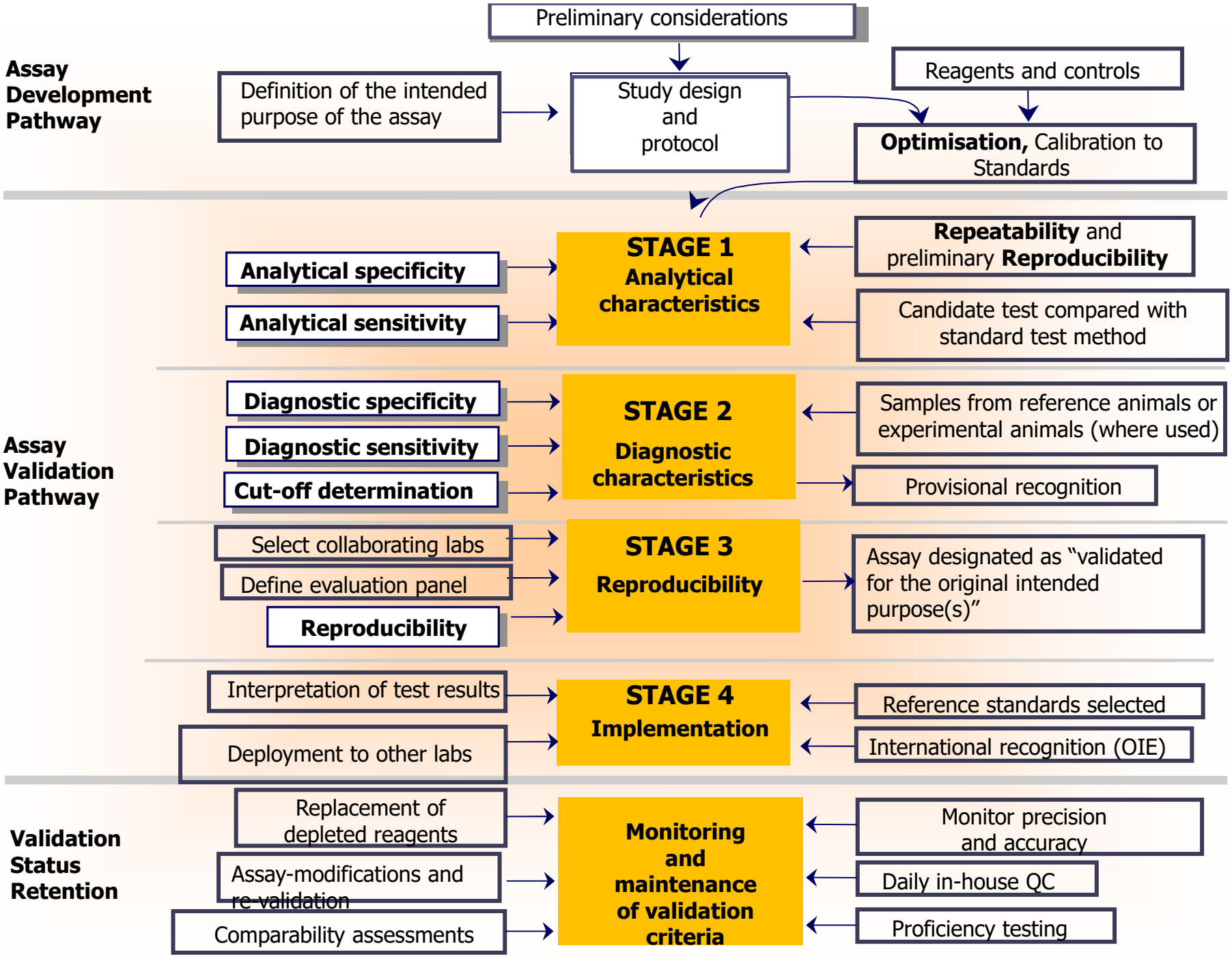
Assays applied to individuals or populations have many purposes, such as aiding in:

- documenting freedom from disease in a country or region,
- preventing spread of disease through trade
- contributing to eradication of an infection from a region or country,
- confirming diagnosis of clinical cases,
- estimating infection prevalence to facilitate risk analysis,
- identifying infected animals toward implementation of control measures,
- and classifying animals for herd health or immune status post-vaccination.

A single assay may be validated for one or more intended purposes by optimising its performance characteristics for each purpose.

International and national reference standards Calibration of the assay to standard reagents

- Ideally, OIE or other international reference standards, containing a known concentration or titre of analyte, are the reagents to which all assays are standardised.
- Such standards are prepared and distributed by OIE Reference Laboratories or other international reference laboratories.
- National reference standards are calibrated by comparison with an international reference standard whenever possible; they are prepared and distributed by a national reference laboratory.
- These standards are highly characterised through extensive analysis, and preferably the methods for their characterisation, preparation, and storage have been published in peer-reviewed publications


Assay Development Pathway
Preliminary considerations
Definition of the intended purpose of the assay
Study design and protocol
Reagents and controls
Optimisation, Calibration to Standards
Assay Validation Pathway
Analytical specificity
Analytical sensitivity
STAGE 1
Analytical characteristics
Repeatability and preliminary Reproducibility
Candidate test compared with standard test method
Diagnostic specificity
Diagnostic sensitivity
Cut-off determination
STAGE 2
Diagnostic characteristics
Samples from reference animals or experimental animals (where used)
Provisional recognition
Select collaborating labs
Define evaluation panel
Reproducibility
STAGE 3
Reproducibility
Assay designated as "validated for the original intended purpose(s)"
Interpretation of test results
Deployment to other labs
STAGE 4
Implementation
Reference standards selected
International recognition (OIE)
Validation Status Retention
Replacement of depleted reagents
Assay-modifications and re-validation
Comparability assessments
Monitoring and maintenance of validation criteria
Monitor precision and accuracy
Daily in-house QC
Proficiency testing

Validation criteria

- **Optimisation** is the process by which the most important physical, chemical and biological parameters of an assay are evaluated and adjusted to ensure that the performance characteristics of the assay are best suited to the intended application.
- **Repeatability** is the level of agreement between results of replicates of a sample both within and between runs of the same test method in a given laboratory. Repeatability is estimated by evaluating variation in results of replicates.
- **Reproducibility** is the ability of a test method to provide consistent results, as determined by estimates of precision, when applied to aliquots of the same samples tested in different laboratories, preferably located in distinct or different regions or countries using the identical assay (protocol, reagents and controls).
- **Analytical specificity** is the ability of the assay to distinguish the target analyte (e.g. antibody, organism or genomic sequence) from non-target analytes, including matrix components .
- The limit of detection (LOD) is a measure of the **analytical sensitivity** of an assay. The LOD is the estimated amount of analyte in a specified matrix that would produce a positive result at least a specified percent of the time.

Summary

- Sampling camel is the biggest thread for laboratory diagnostic confirmation
- Serologic tests form the basis for surveillance in most countries ELISA, SN, AGID, CF.
- Virus/bacteria isolation is needed to determine the pathogenicity of field isolates
- Molecular diagnostics (rRT-PCR) are rapidly replacing conventional isolation procedures
- Need of collaboration between OIE Reference Laboratories and National labs for diagnosis techniques validation for diseases of camels


Thank you
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