


Diagnostic protocols and tests



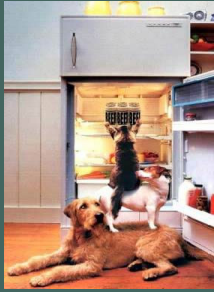
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OIE Collaborating Centre for Training in Integrated Livestock and Wildlife Health and Management Laboratory Diagnostics

Sampling is a tool, to make it meaningful is a team effort





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The team

- Close co-operation between different disciplines
 - **Field:** veterinarians (state, private), para-veterinarians, animal owners, game wardens, biologists, etc.
 - **Laboratory:** pathology, clinical pathology, bacteriology, virology, parasitology, toxicology, etc.
 - **Biostatistician**



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The team

- Involvement of laboratory in sampling plan
- Consult laboratory for most appropriate diagnostic tools and samples
- Laboratory network enhances capacity in the region



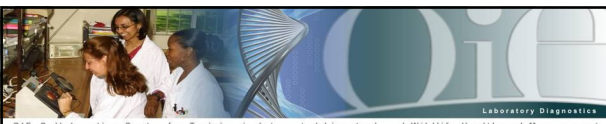
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The ideal world

- Field:
 - Fresh carcass
 - Typical clinical signs
 - Comprehensive history
- Laboratory:
 - In close proximity
 - Capacity in all disciplines
 - Well equipped incl. cutting edge technology
 - Fast-tracking service

The real world

- Field:
 - No/decomposed carcass
 - Clinical signs variable and/or non-specific
 - No history
- Laboratory:
 - Samples take several days to reach laboratory
 - Basic and few advanced procedures
 - Poorly equipped
 - Limited flexibility in working hours



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Sample handling and packaging

- Use reputable courier/transport
- In time
- Maintenance of cold chain

Laboratory Diagnostics

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Sample handling and packaging: The Do's:

3-layer packaging: 1. Leak proof containers




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Laboratory Diagnostics

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Sample handling and packaging: The Do's

2. Maintain temperature
e.g. Place samples in polystyrene containers with freezer blocks to maintain at 4°C (not frozen)



Laboratory Diagnostics

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Sample handling and packaging: The Do's

- Good insulation packaging
- Place submission form in separate plastic bag
- Avoid mixing formalin bottles with samples for culture





Sample handling and packaging: The Do's

3. Rigid outer packaging





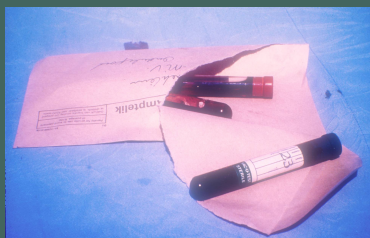
Sample handling and packaging: The Do's

- Regulations for the Transportation of Infectious Substances
- General Requirements for diagnostic specimens ("Biological Substance, Category B" must be marked on the outer packaging adjacent to the diamond-shaped mark) - P.I. 650 - for UN 3373

Guidance on regulations for The Transport of Infectious Substances
September 2005
WHO/CDS/CSR/LYO/2005.22
Communicable Disease Surveillance and Response



Sample handling and packaging: The DO NOT's





**Sample handling and packaging:
The DO NOT's**





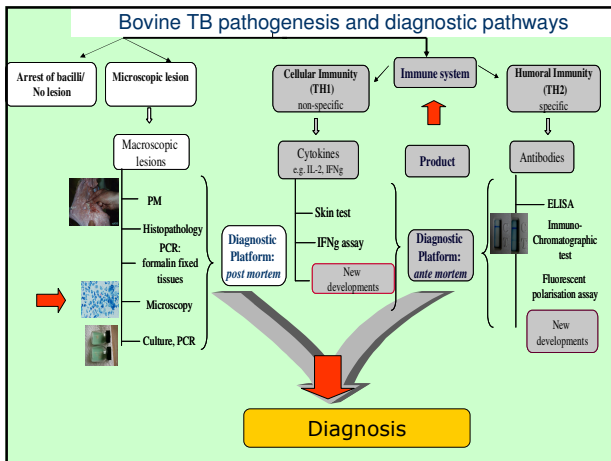
**Sample handling and packaging:
The DO NOT's**





**Diagnostic procedures
From the field to the laboratory**

The fresher and better the samples the higher the quality of the laboratory analyses and the diagnostic outcome



Diagnostic procedures: From the field ...

- Intradermal tuberculin test
 - Cattle and buffalo
 - Dose and interpretation
 - Only reliable as a herd test
 - High risk and costs in wildlife

Optimised BTB diagnosis: IDT & Interferon gamma assay (IFN- γ)

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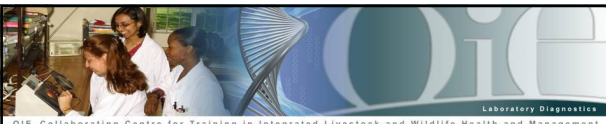
Incubate o/n at 37C

IFN γ ELISA to measure cellular immune response in response to tuberculin



Diagnostic procedures From the field to the laboratory

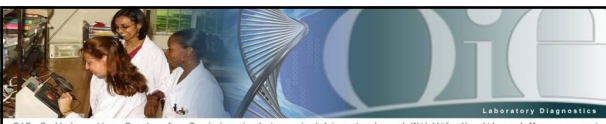
The fresher and better the samples the higher the quality of the laboratory analyses and the diagnostic outcome



Sample submission

- Serum
 - Collected in tubes without anticoagulant (red top)
 - Detection of antibodies to infectious agents
 - Best used on herd basis
- Maintenance of cold chain

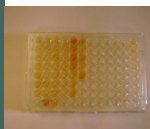
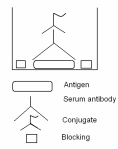




Serology - tests

Formats:

- ELISA (antigen or antibody)
- Immuno Fluorescent Antibody assay
- Virus neutralization test
- Rose Bengal Plate agglutination (brucellosis)
- Complement Fixation test
- Fluorescent polarization assay
- Rapid tests (pen-side tests)



Agricultural Research Council - Diagnosis of bacterial diseases - Microsoft Internet Explorer

Address: http://www.arc.agric.za/home.asp?pid=495

Animal side test

- **Rose Bengal Test (RBT)**
This is a simple but sensitive screening test which is carried out on all sera. Equal volumes (25 µl) of serum and antigen (killed *Brucella abortus* organisms) are mixed in a plate for 4 minutes. In positive cases an agglutination reaction will take place between the antibodies in the serum and the antigen which can be seen as a ring in the well of the plate.
- **Serum Agglutination Test (SAT)**
This is also a screening test involving an agglutination reaction. The serum is serially diluted in 4 wells of a microplate (1:10, 1:20, 1:40, and 1:80) before the antigen is added. This gives an indication of the number of antibodies in the serum and hence how positive the sample is. A reaction that only occurs in the first well indicates few antibodies. A reaction that extends to the fourth well indicates many antibodies because even after diluting the serum 1:80 there are still sufficient antibodies to cause an agglutination reaction.
- **Complement Fixation Test (CFT)**

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Laboratory Diagnostics

Sample submission

- Culture
 - Tissue: 5 – 10grams
 - Swabs
 - Fluids
 - Blood (whole blood) - if bacteraemia or viraemia is suspected
- Collect aseptically
- Use sterile containers
- Use transport medium (commercial) where applicable

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Laboratory Diagnostics

Anaerobic culturing

Avoid contact with air by:

- Sample in syringe: expel air and cap needle
- Fluids can be injected through the cap of a vacutainer (pink-capped)
- Tissue samples - large to fill container/place in anaerobic transport To reach the laboratory as soon as possible
- Intestinal content in tied off intestine

Avoid: swabs & discharges

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Sample submission

- Histopathology
 - Direct pathogen detection
 - Small tissue blocks
 - Use sharp knife (no scissors)
 - Place in 10% buffered formalin
 - Do not freeze

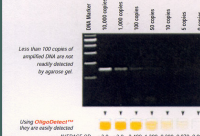


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Sample submission

- Molecular tests
 - Direct pathogen detection
 - Tissue, fluids, faeces, blood
 - PCR, Reverse Line Blot



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Thank you!