Validation of Diagnostic Tests for Wildlife

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Brucella melitensis is absent

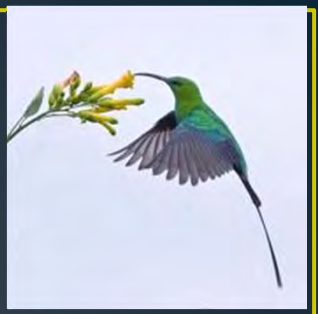
Serology (i-ELISA) for *Brucella melitensis*: 10/25 animals test positive

Manufacturer's specifications:

- Method: Indirect ELISA
- Species: Ruminants
- Specimens: Bovine, ovine and caprine serum

Test not validated for the target species!

What is test validation?



- Process that determines the fitness of an assay for an intended purpose
- The assay is subjected to a validation pathway in which

the assay's analytical and diagnostic performance is determined.

Note:

Test validation depends on compliance with

Quality management in veterinary testing laboratories (e.g. ISO17025)

Why test validation?

Confidence in test results obtained

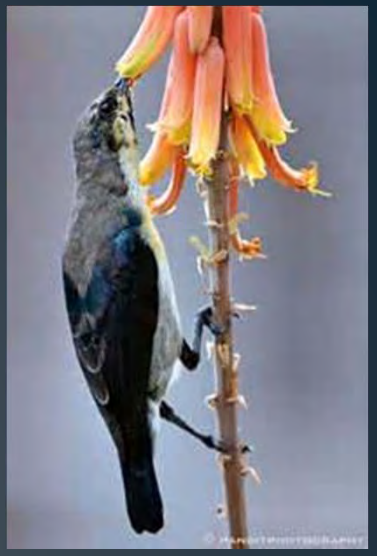
Ensure quality of the test results

International recognition

Diagnostic tests

Fitness for (intended) purpose

The capacity of a positive or negative test result to predict accurately the infection or exposure status of the animal or population of animals is the ultimate consideration of assay validation.



Wildlife/livestock/human interface


75% of all diseases which have
emerged in the last two decades are of
wildlife origin
NBO is waiting for us

Diagnostic testing of wildlife becoming ↑ important **because of** diseases which can have an impact on

- wildlife populations and biodiversity
- Health of humans
- Health of domestic animals

VALIDATION

Approach adopted for test validation in domestic animals presents challenges for use in wildlife

Develop approach applicable to wildlife

Intended purposes (wildlife)

1) Screening wildlife populations for the presence of infectious agents, for example:
   a) for surveillance (e.g. early detection, evaluation of trends in prevalence or incidence)
   b) to estimate prevalence of infection or exposure
2) Screening or testing vectors or environmental samples for the presence of infectious agents
3) Confirming a diagnosis of suspect or clinical cases (includes confirmation of positive results from a screening test)
4) Certifying freedom from infection or presence of the agent in individual animals or products, for

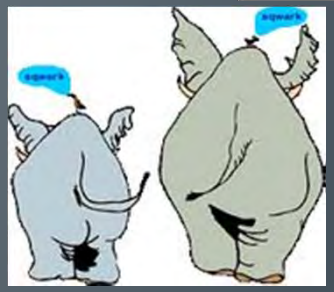
a) movement or translocation
b) human consumption

5) Monitoring of the geographical distribution and prevalence changes due to management interventions (including determining immune status of individual animals or populations)
6) Studying agent, host and environment factors associated with disease occurrence

The road to test validation

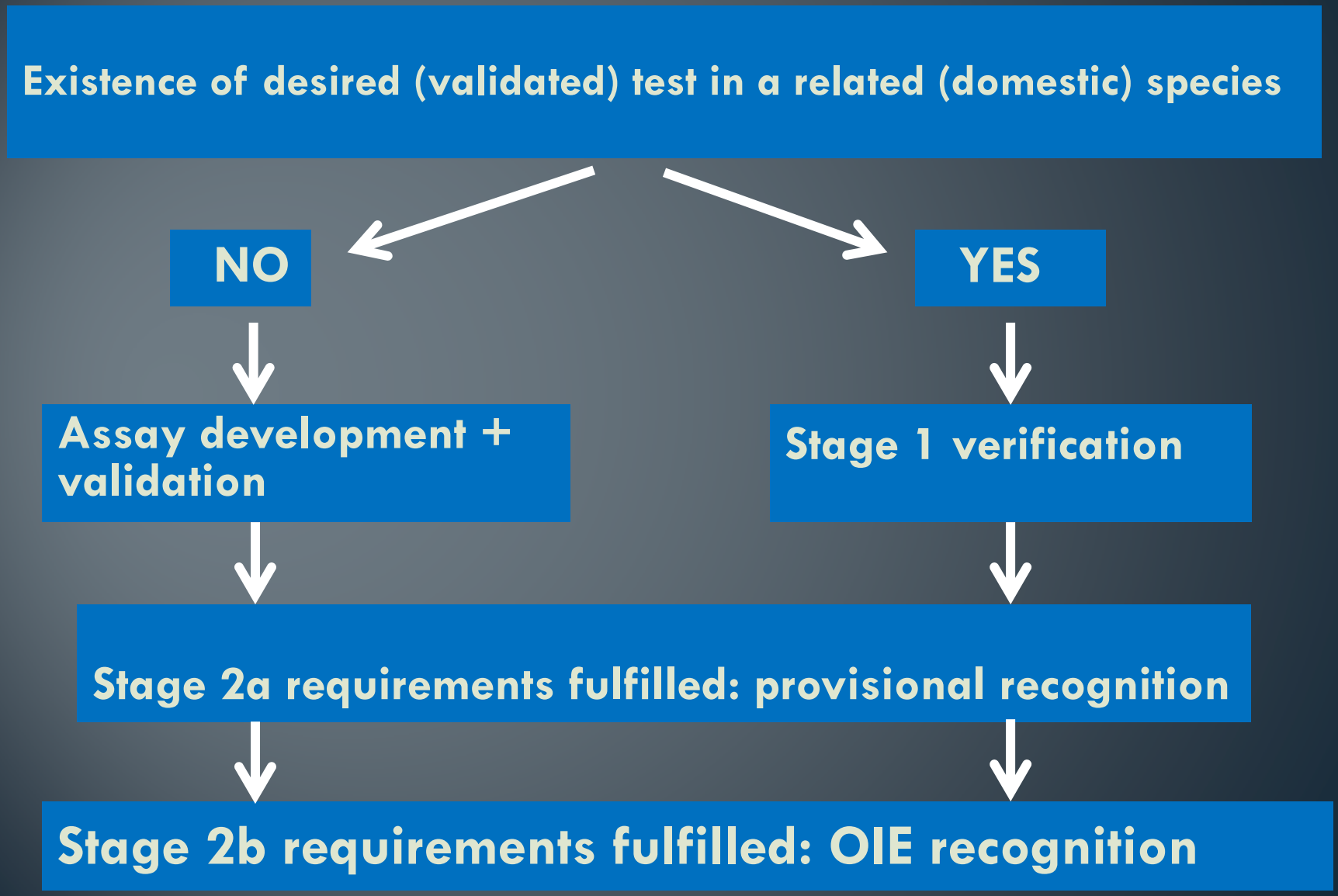
Characteristics used in validation

- Definition of the intended purpose(s)
- Optimization
- Standardisation
- Analytical sensitivity
- Analytical specificity
- Diagnostic sensitivity
- Diagnostic specificity
- Thresholds (cut-offs)
- Reproducibility
- Fitness for intended purpose(s)

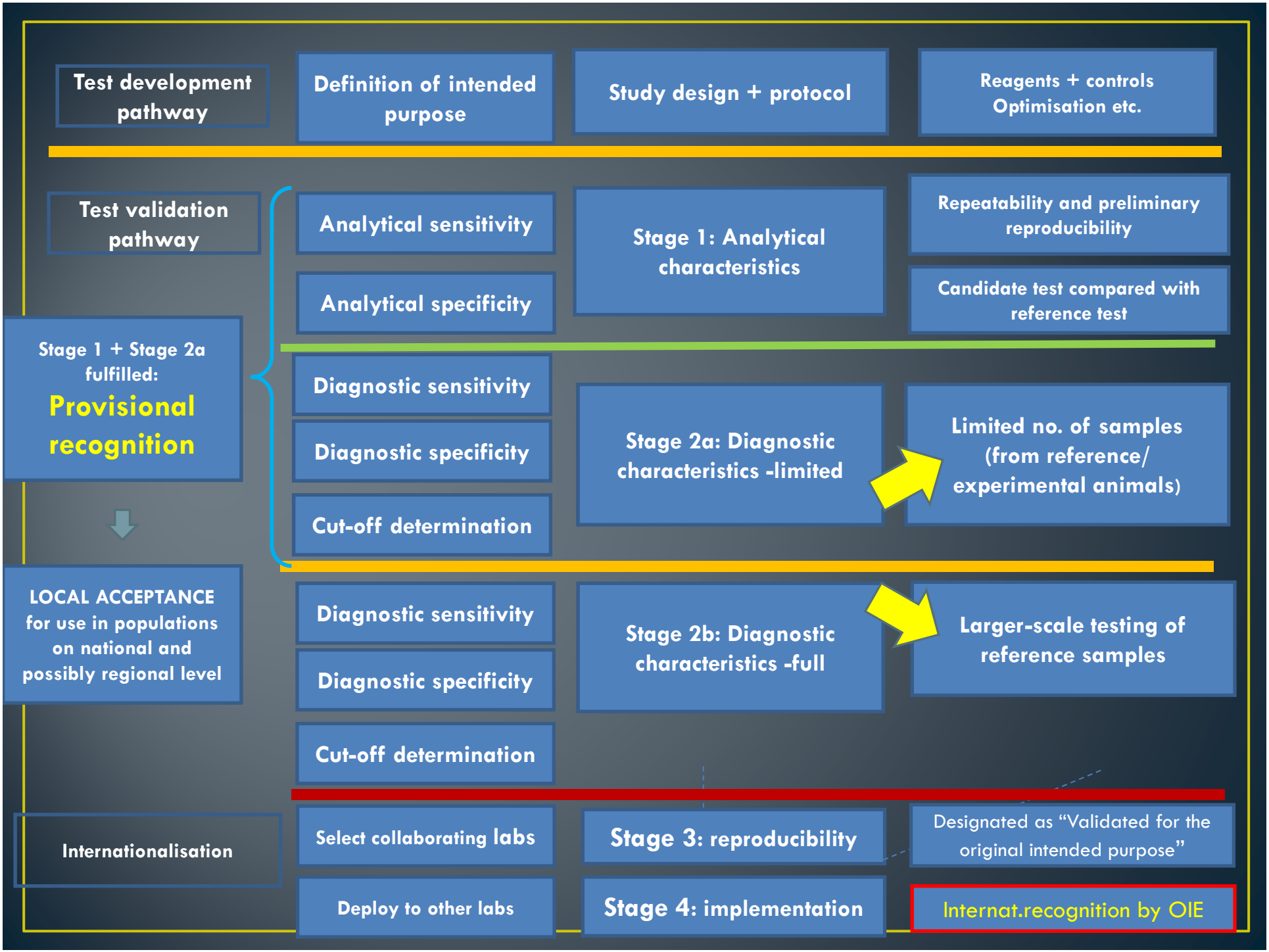



Assay development pathway
Definition of intended purpose
Study design + protocol
Reagents + controls
Optimisation
Analytical sensitivity
Analytical specificity
Stage 1: Analytical characteristics
Repeatability and preliminary reproducibility
Candidate test compared with reference test
Assay validation pathway
Diagnostic sensitivity
Diagnostic specificity
Cut-off determination
Stage 2: Diagnostic characteristics
Samples from reference animals
Samples from experimental animals
Select collaborating labs
Stage 3: Reproducibility
Assay designated as "validated for the original intended purpose"
Deploy to other labs
Stage 4: Implementation
Reference standards selected
Internat.recognition by OIE
Validation Status Retention
Assay modifications and re-validation
Monitoring and maintenance of validation criteria
Daily in-house QC
Proficiency testing

2 scenarios: diagnostic tests for wildlife




WELCOME
Provisional recognition of diagnostic tests
ON BOARD


Test development pathway
Definition of intended purpose
Study design + protocol
Reagents + controls Optimisation etc.
Test validation pathway
Analytical sensitivity
Analytical specificity
Stage 1: Analytical characteristics
Repeatability and preliminary reproducibility
Candidate test compared with reference test
Stage 1 + Stage 2a fulfilled:
Provisional recognition
Diagnostic sensitivity
Diagnostic specificity
Cut-off determination
Stage 2a: Diagnostic characteristics -limited
Limited no. of samples (from reference/ experimental animals)
LOCAL ACCEPTANCE for use in populations on national and possibly regional level
Diagnostic sensitivity
Diagnostic specificity
Cut-off determination
Stage 2b: Diagnostic characteristics -full
Larger-scale testing of reference samples
Internationalisation
Select collaborating labs
Deploy to other labs
Stage 3: reproducibility
Stage 4: implementation
Designated as "Validated for the original intended purpose"
Internat.recognition by OIE

Validation pathway Chapter 1.1.5.	Pathway 1: No validated test in related species*	Pathway 2: Validated test in related species*
Stage 1	Stage-1 verified in new target species	Stage-1 verified in new target species
Analytical specificity	Yes	Yes
Analytical sensitivity	Yes	Yes
Repeatability	Yes	No
Reproducibility (preliminary)	Yes	No
Stage 2	Stage 2a (Provisional recognition)	Stage 2a (Provisional recognition)
Diagnostic sensitivity	Yes (minimum of 30 positive reference samples)	Yes (minimum of 10 positive reference samples)
Diagnostic specificity	Yes (minimum of 30 negative reference samples)	Yes (minimum of 10 negative reference samples)
Cut-off determination	Yes (total of 60 samples)	Yes (total of 20 samples)
Reference sample description	Yes	Yes
	Stage 2b	Stage 2b
Diagnostic sensitivity	Yes	Yes
Diagnostic specificity	Yes	Yes
Cut-off determination	Yes	Yes
Reference sample description	Yes	Yes
Stage 3	Stage 3	Stage 3
Reproducibility	Yes	Yes
Repeatability	Yes	Yes
Stage 4	Stage 4	Stage 4
Predictive values (populations)	Yes	Yes

Sample size for full validation: stage 2b - *Domestic animals*

Table 1. Theoretical number of samples from animals of known infection status required for establishing diagnostic sensitivity (DSe) and specificity (DSp) estimates with known confidence

	2% error allowed in estimate of DSe and DSp			5% error allowed in estimate of DSe and DSp		
Estimated	Confidence			Confidence		
DSe or DSp	90%	95%	99%	90%	95%	99%
90%	610	864	1493	98	138	239
92%	466	707	1221	75	113	195
94%	382	542	935	61	87	150
95%	372	456	788	60	73	126
96%	260	369	637	42	59	102
97%	197	279	483	32	45	77
98%	133	188	325	21	30	52
99%	67	95	164	11	15	26

Percent error allowed in the estimate of DSe or DSp = 2% in the left panel and 5% in the right panel. For the number of samples required for 1%, 3%, and 4% error margin in the estimate of DSe and DSp, multiply the number of samples in the left panel of the table by a factor of 4.0, 0.44, and 0.25, respectively.

Sample size for full validation: stage 2b
- *Wildlife*

- Absolute numbers of samples may initially be lower
- Calculated error margins will be wider
- Increase in uncertainty in test performance criteria

No. positive reference samples	No. positive	DSe (%)	Approximate error margin on estimate of DSe	95% exact binomial confidence interval for DSe (%)
140	126	90	± 0.05	83.8 – 94.4
100	90	90	± 0.06	82.4 – 95.1
60	54	90	± 0.08	79.5 – 96.2
30	27	90	± 0.10	73.5 – 97.9
10	9	90	± 0.18	55.5 – 99.7

Sample size for full validation: stage 2b

- Wildlife

Recommendations to obtain required certainty:

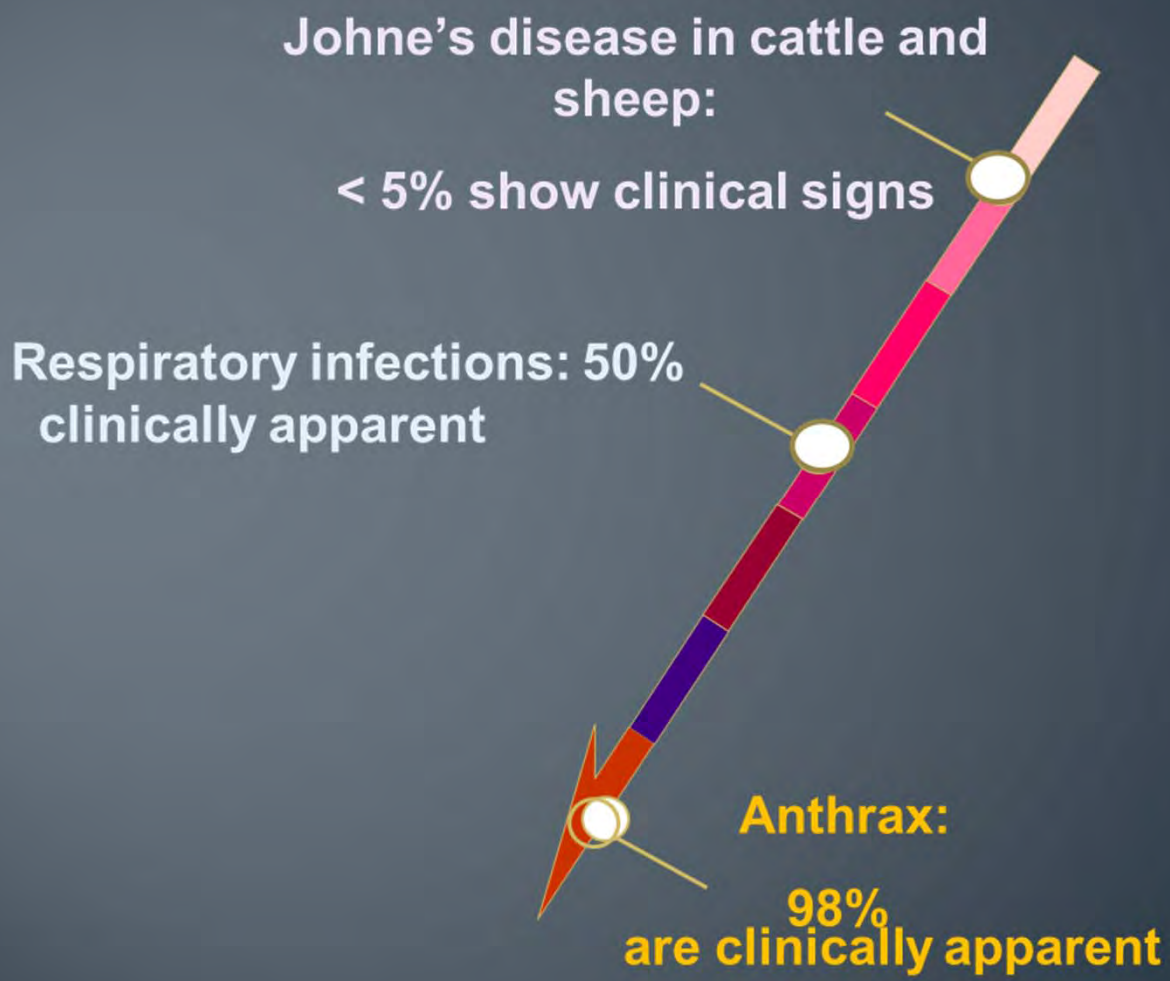
- Combine data from multiple laboratories
- Build validation database **over time**

Recommendations cont'd:

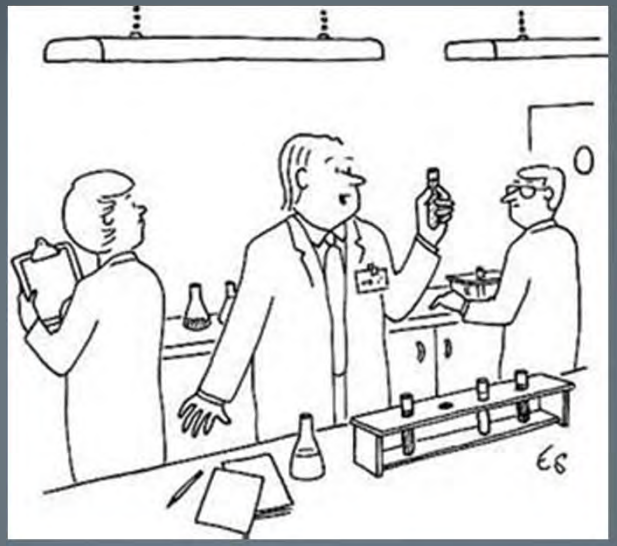
Using representative samples of the target condition is of greater importance than sample size.

- Why?
- Achieves an unbiased (and practically useful) estimate of DSe and DSp that will stand up to scrutiny over time.

How target conditions influence Dse



Common constraints



"This test is validated" ≠ **"This test is fit for purpose"**

Test constraints

Indirect tests

Require species specific immunological reagents (antibodies)

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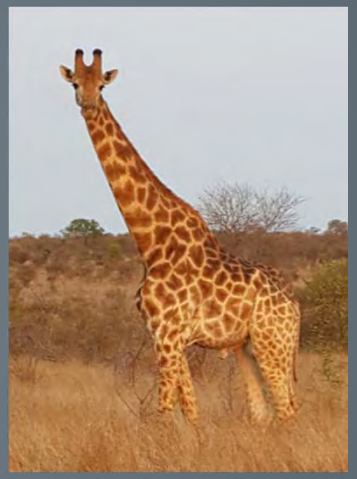

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Test constraints

A bit of good news…

- Test methods for direct pathogen detection are generally not species-specific:

However, consider the following:

Species variations in pathogen proliferation rate may affect the amount and distribution of the pathogen in the body

Challenges in test validation

- It is deemed useful and necessary to validate appropriate tests for a range of sample condition criteria such as different sample types, changes in detectability over time, under different storage temperatures, during autolysis, etc.

Test constraints –
Samples from wildlife

- Accessibility to diagnostic reference samples (adequate numbers, volume)

  Positive reference samples

  pooling small quantities of samples from few infected individuals

  Dilution of a strongly positive sample to create a series of samples with different concentrations

  Negative reference samples

Test constraints –
Samples from wildlife

Quality of reference samples

- Opportunistic screening of dead animals is an effective way of monitoring wildlife populations for infectious agents

- Disadvantage: compromised sample integrity (cross-contamination, autolysis)

- What to do?
- Ensure maximum utility of scarce samples
- Determine suitability for test validation, describe as (good, poor, autolysed)

Test constraints –

Samples from wildlife

Only limited sample information available:

What is essential?

a) the precise host species,

b) specimen type

c) geographical location with reference to known disease free or infected areas/regions,

d) the date of sample collection

e) Wherever possible, information on sex, age category (juvenile, sub-adult, adult), absence or presence of clinical signs, and a description of the signs will add value.

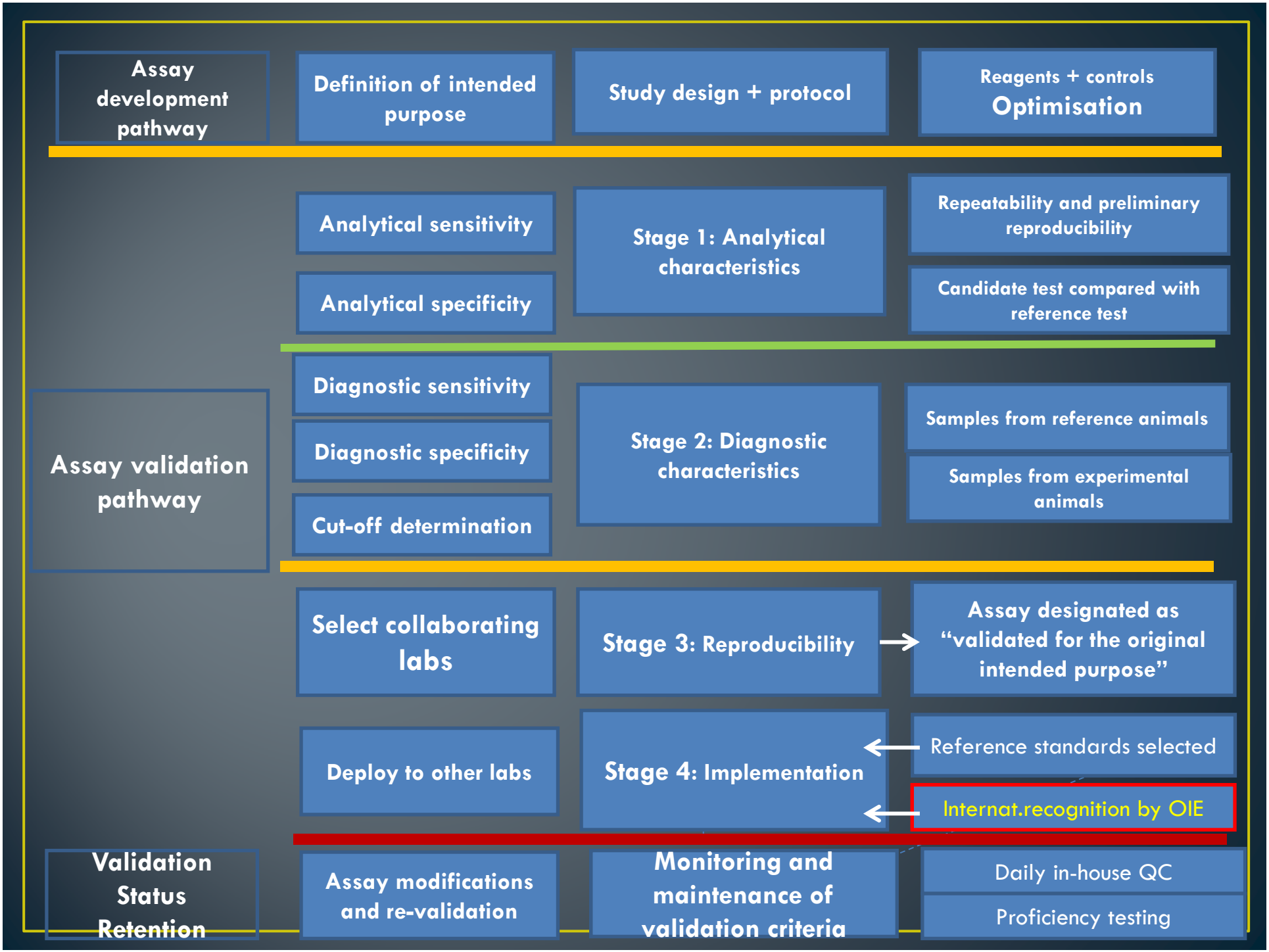
In conclusion, …

- Validation of diagnostic tests used in wildlife
  - has become important
  - has been made feasible due to the recommendation of the principle of provisional recognition

However, …

Non-validated tests can be worthwhile to use in a scientific approach (after all this is the way in which we improve existing methodologies)


Questions?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