LOHMANN
BREEDERS

LOHMANN BREEDERS

LOHMANN BREEDERS

The specialist for layer breeding

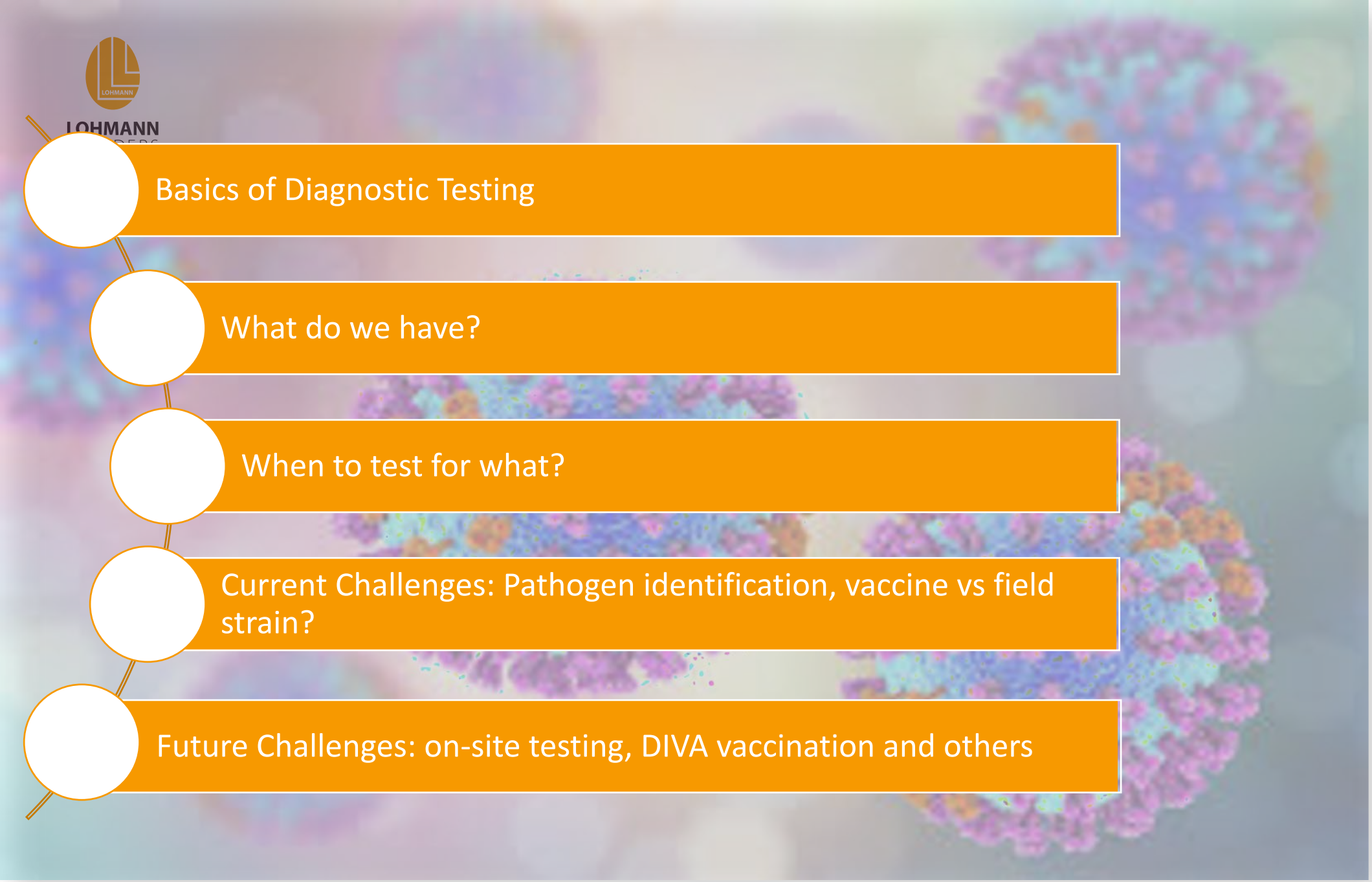
Current & future challenges of poultry diagnostics

Dr. Matthias Voss; Veterinary Scientific Director

ECO Asia Poultry
Online Conference 2021

BREEDING FOR SUCCESS…TOGETHER

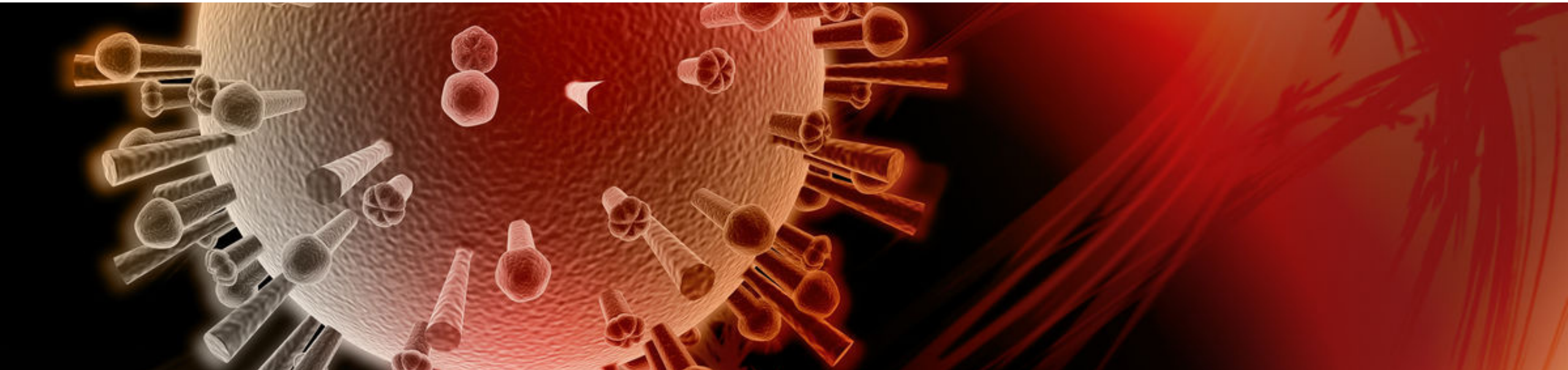
LOHMANN BREEDERS



- Basics of Diagnostic Testing
- What do we have?
- When to test for what?
- Current Challenges: Pathogen identification, vaccine vs field strain?
- Future Challenges: on-site testing, DIVA vaccination and others

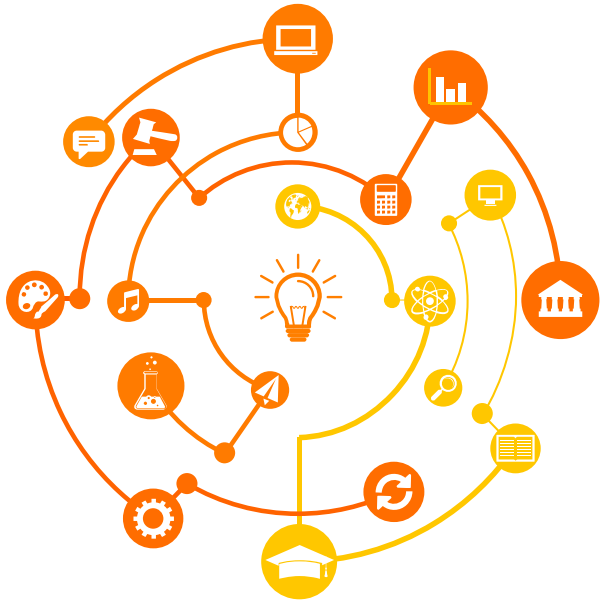
LOHMANN
BREEDERS

Basics of Diagnostic Testing



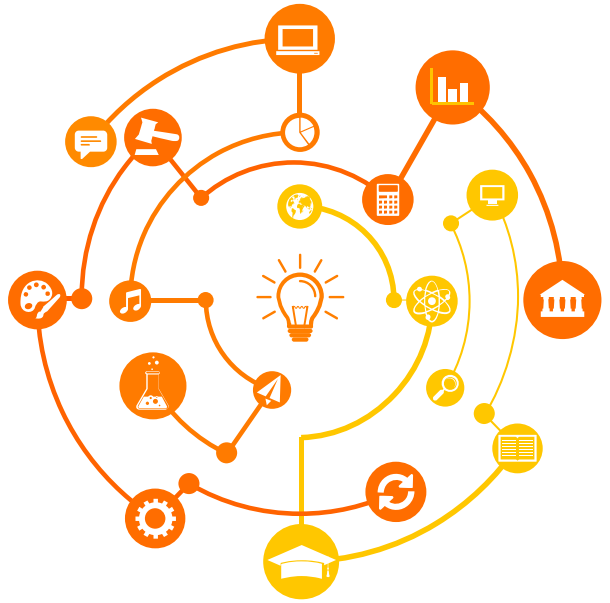
Reasons for diagnostic testing

- mandatory / regulatory testing:
  - disease control programs, trade (e.g., Salmonella, Avian Influenza, Mycoplasma)
- Vaccination monitoring:
  - evaluation of efficacy of vaccination program, need for adaptation, new pathogens in the field
- Clinical health problems:
  - mortality, respiratory, egg production drops
- hygiene monitoring:
  - Farms, hatchery



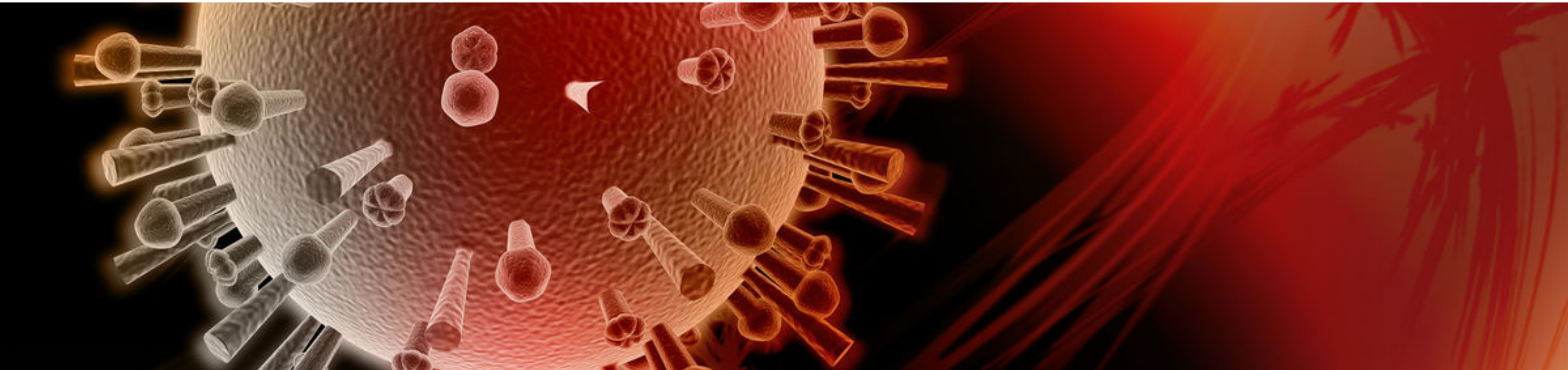
Requirements for diagnostic testing

- Rapid:
  - Immediate identification of certain highly contagious disease, important to limit further transmission (Avian Influenza! Newcastle!)
- Sensitive and specific:
  - Tests need to show high sensitivity by acceptable specificity (false positives / false negatives)
- Affordable:
  - Test must be affordable in relation to the diagnostic outcome; ad the end in most cases the farmer must pay; cost benefit



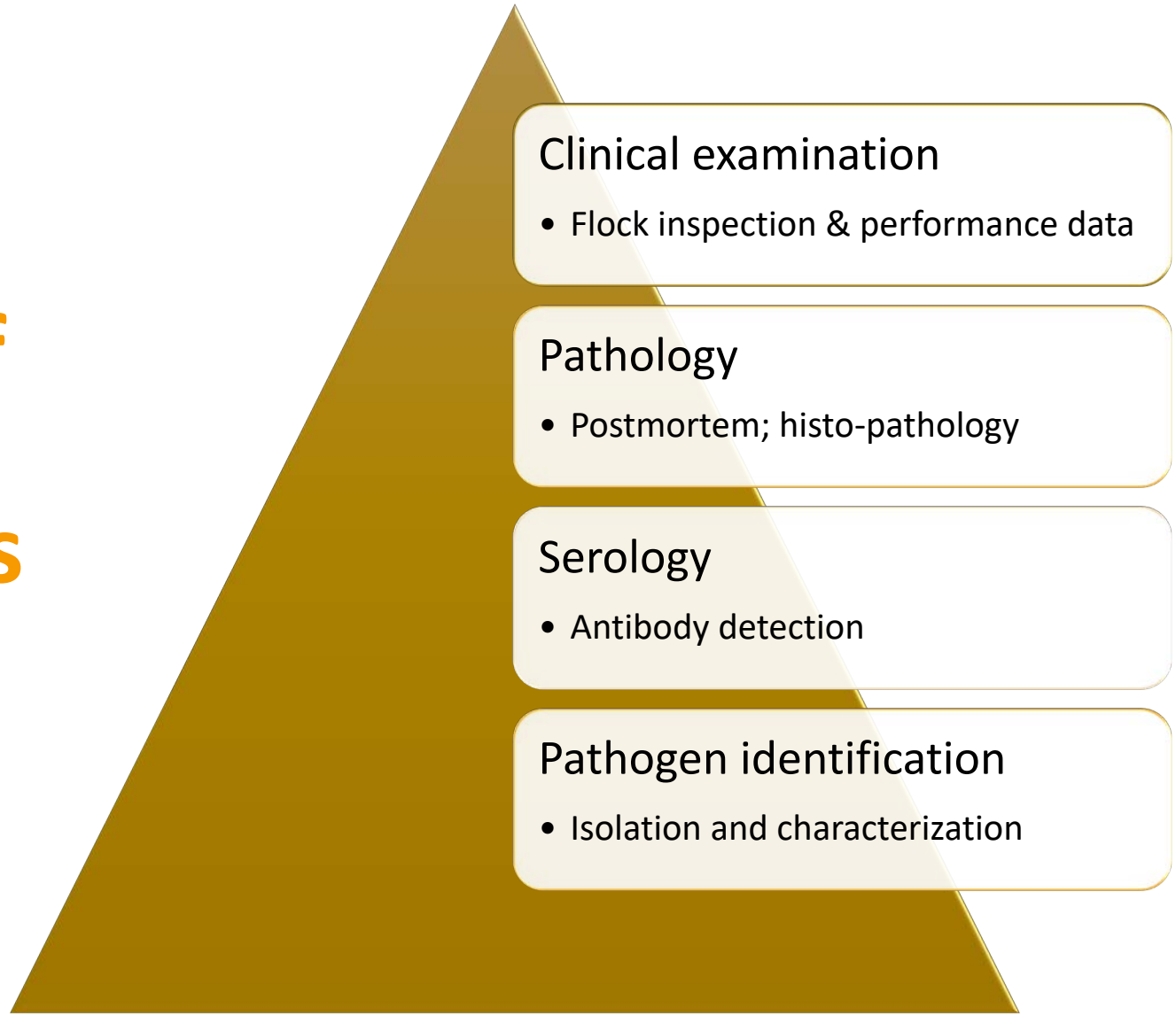
LOHMANN
BREEDERS

What do we have?



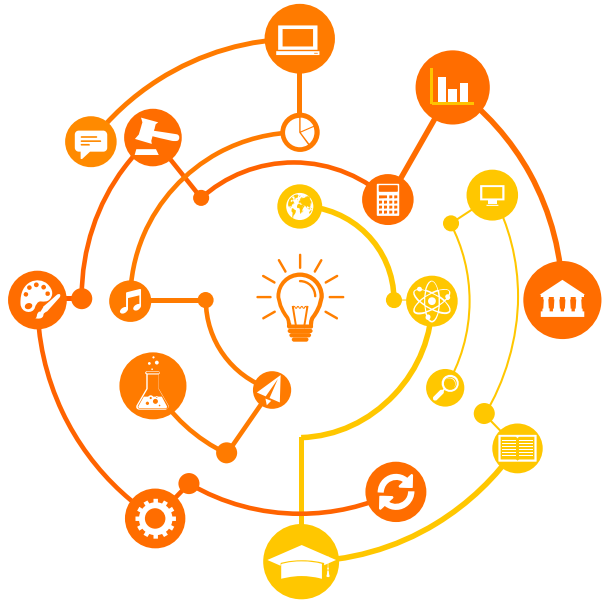
LOHMANN
BREEDERS

Pyramid of Poultry Diagnostics



Serological Methods used in Poultry

- Rapid Plate Agglutination (RPA)
- Agargel Precipitation Test (AGPT)
- Hemagglutination Inhibition Test (HI)
- ELISA
- Virus Neutralisation (VN) Test
- Serum Neutralisation (SN) Test
- Immunofluorescence

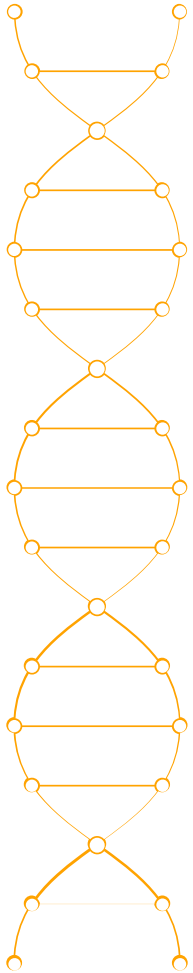


Rapid Plate Agglutination (RPA)

Could be used for	Advantage	Disadvantage
Mycoplasma (Mg, Ms)	Easy and rapidly to perform	• Quality of antigens available in the market • False positive reactions especially after use of inactivated vaccines • Less sensitive as some of the ELISA tests?
S. gallinarum / pullorum	Easy and rapidly to perform	• Quality of antigens available in the market

LOHMANN BREEDERS

Agargel Precipitation Test (AGPT)

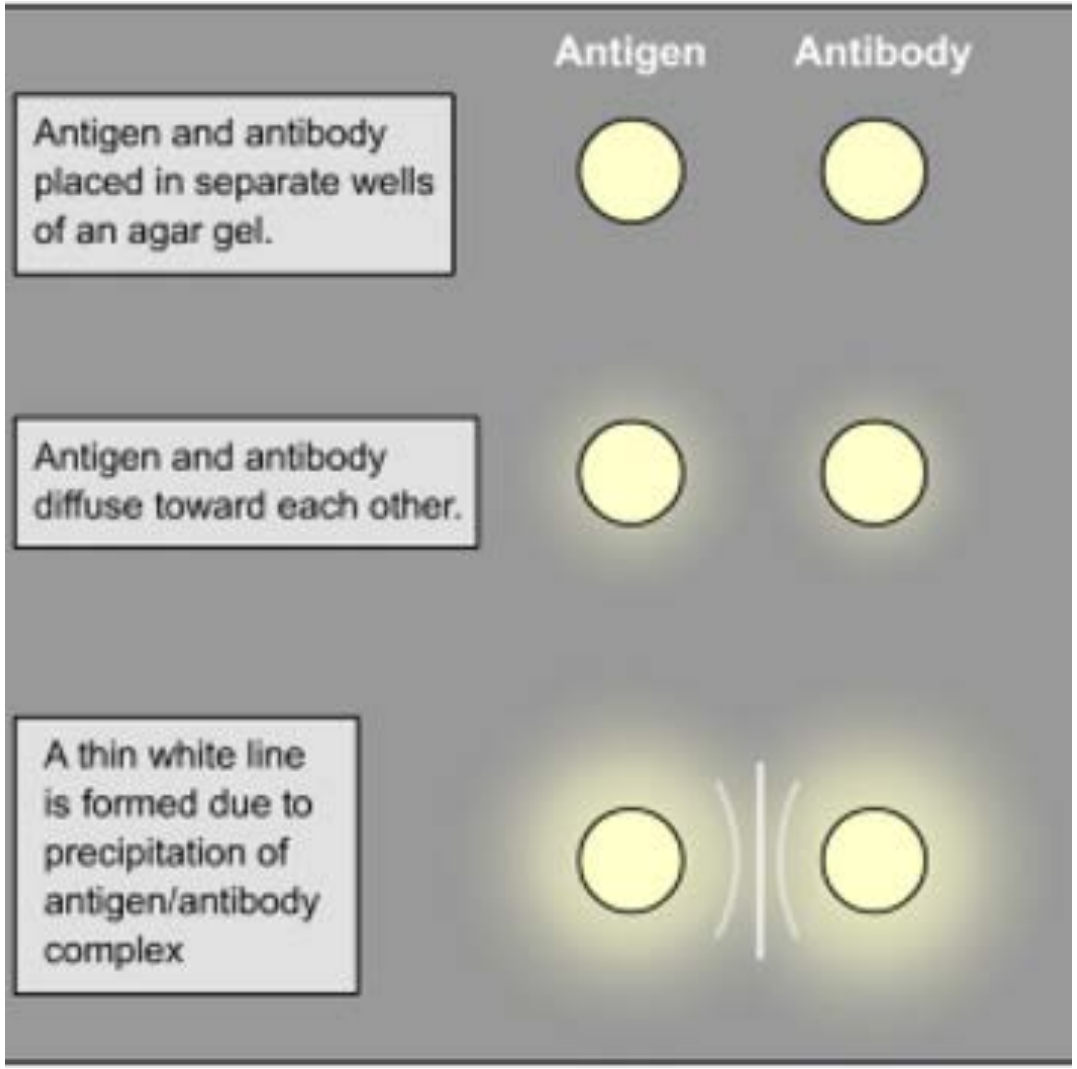


Could be used for	Advantage	Disadvantage
Infectious Bronchitis	• Easy to perform • Detects contact of a flock with live virus (both vaccine or field virus) • Group specific	• Only yes / no answer • No actual antibody titer • Not suitable to differentiate between different IB strains
Fowl Adeno Virus (FAdV)	• Easy to perform • Group specific (if antigens used are suitable)	• Only yes / no answer • No actual antibody titer • Not suitable to differentiate between different FAdV strains
Marek's	• Only routine test available for the detection of antibodies	• Not relevant for protection (only for testing SPF flocks)

LOHMANN
BREEDERS

Agar Gel Precipitation: AGP

- easy to perform
- qualitative results: yes / no
- group specific antigens
- not suitable to differentiate between different strains (e.g. IB, AI)
- AGP sometimes mandatory for trade purpose

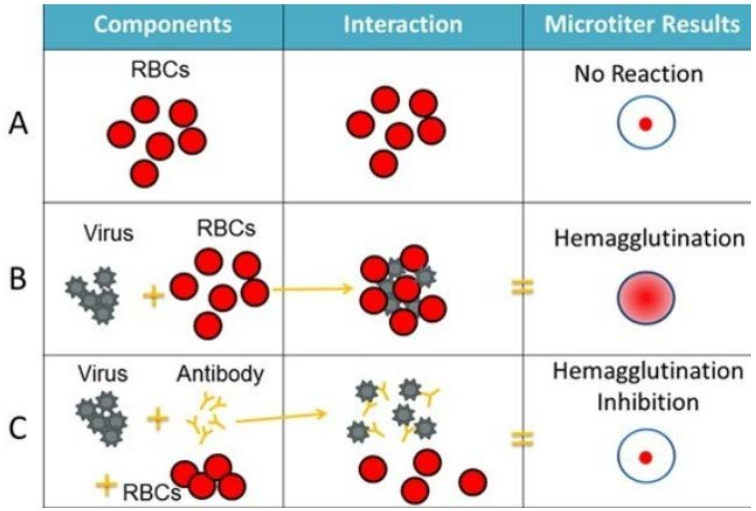


Haemagglutination Inhibition Test (HI)

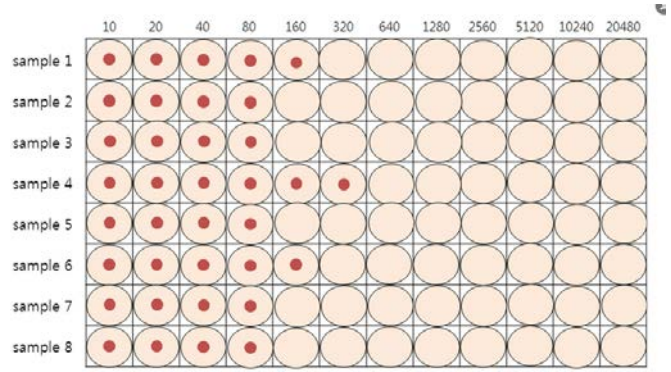
Could be used for	Advantage	Disadvantage
Infectious Bronchitis	• Easy to perform • Serotype-specific	• more cross-reactions compared to VN test
Newcastle Disease	• Easy to perform • Even live vaccines could be used as antigen • Used if ELISA technology is not available	• Correlation between HI and ELISA some times difficult to interpretate
Egg Drop Syndrome	• Easy to perform	• Non if antigen is available
Avian Influenza	• Only way to differentiate between antibodies against different AI viruses	• Only possible for specialist laboratories

LOHMANN
BREEDERS

Hemagglutination inhibition test: HI



- Only with hemagglutinating virus : AI, ND, EDS, IB
- serotype-specific
- live vaccines as antigen used
- specific positive (4 HI units): log2 = 4



HI titer			
sample 1	160	sample 5	80
sample 2	80	sample 6	160
sample 3	80	sample 7	80
sample 4	320	sample 8	80

LOHMANN
BREEDERS

Enzyme linked Immunosorbent Assay

Could be used for	Advantage	Disadvantage
In principle for most Avian Pathogens	• Commercially available for most relevant viral infections • Commercially available for some of the bacterial infections (e.g. Salmonella, Pasteurella)	• Commercial kits relatively expensive • Need certain lab equipment (e.g. photometer, washer) • May show false positive results (back-up test are needed) • Interpretation of results depend very much on the knowledge of the producer of the kits

Vaccination baselines (Layers/Breeders)

Test	Vaccine type	Mean titer range	Wks after vac. to test	Suspect titer infection
IBV	live (H120, MA5)	2000 - 4000	3 - 5 wks	> 6000
	live /H120, 2nd 4/91)	6000 - 10000	3 - 5 wks	> 12000
	inact.	6000 - 17000	5 - 8 wks	
IBD	live, intermediate	2500 - 7000	3 - 5 wks	> 9000
	inact.	7000 - 25000	5 - 8 wks	
NDV	live, La Sota	2000 - 8000	3 - 5 wks	
	inact.	10000 - 25000	5 - 8 wks	
REO	live	2000 - 5000	3 - 5 wks	> 6000
	inact.	7000 - 20000	5 - 8 wks	
ART	live	2000 - 5000	4 - 7 wks	
		7000 - 25000	5 - 8 wks	
AE	live 1 x	5000 - 12000	4 - 6 wks	
ORT	none	negative		> 10000

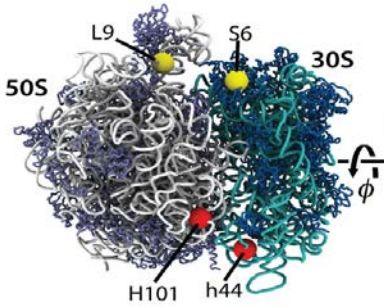
Test: Biocheck, based on two times life priming and one time inactivated boosting at 16 – 18 weeks

Virus Neutralisation (VN)

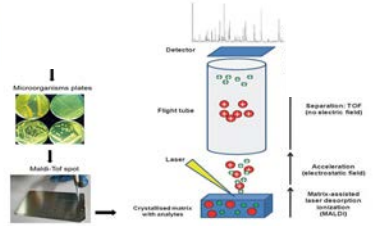
Could be used for	Advantage	Disadvantage
Infectious Bronchitis (IBV)	• Most specific method to test for IB variant antibodies	• Need facilities with tissue culture method • Relatively expensive • Can give only an indication for the strains used for the VN test
Fowl Adenovirus (FAdV)	• Can detect antibodies against serotype 1-12	• Need facilities with tissue culture method • Relatively expensive
Gumboro (IBD)	• Can detect antibodies against serotype 2 virus (only relevant for SPF testing)	• Need facilities with tissue culture method • Relatively expensive

Some Typing Methods for Bacterial Pathogens

- **16s**
  - easy to establish
  - cheap
  - suitable to species level



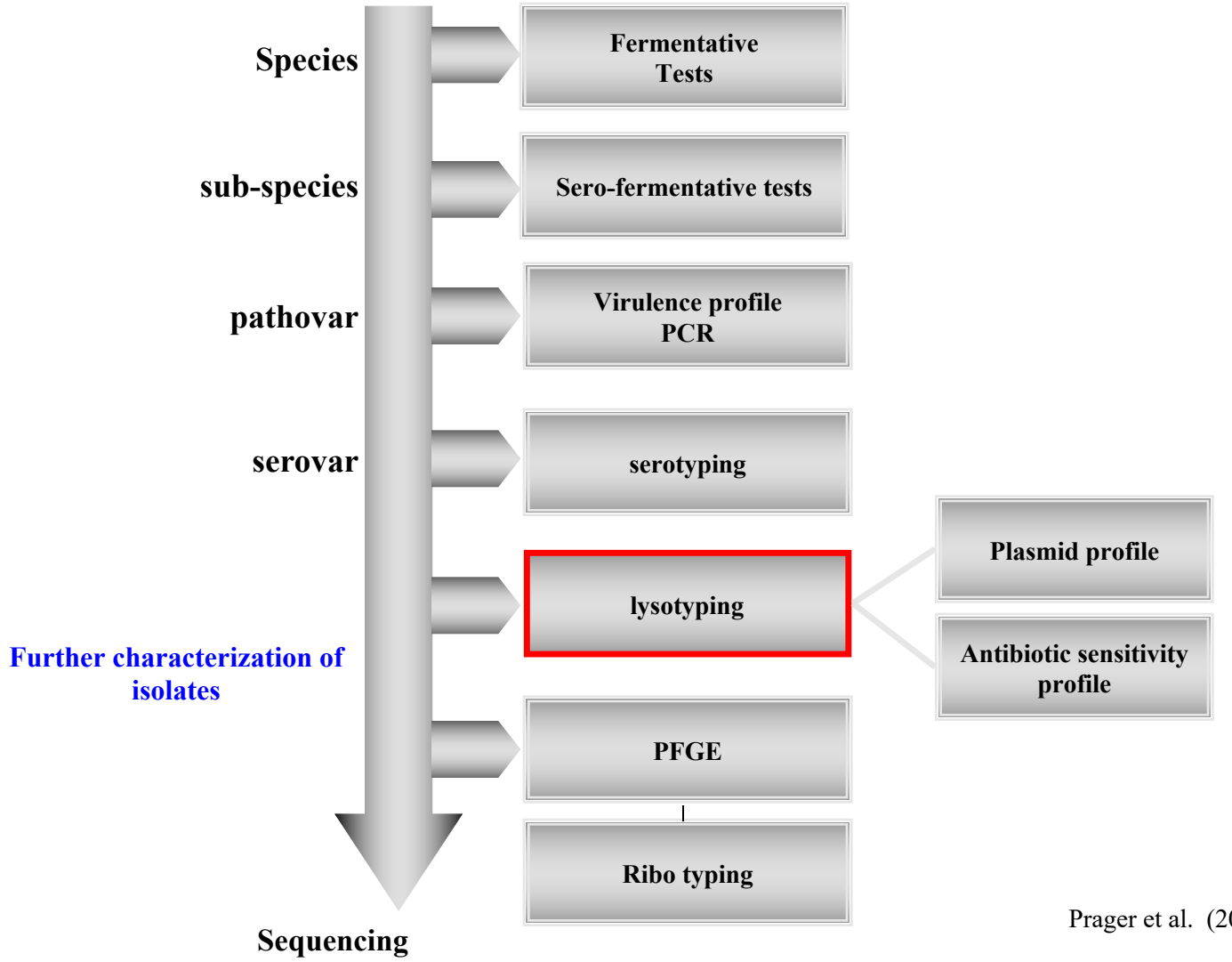
- **Maldi-TOF**
  - require a good database
  - suitable beyond species level



- **NGS**
  - best discrimination power
  - relatively expensive
  - higher operating expense

- **RFLP / MLST**
  - low costs
  - suitable beyond species level
  - not standardisable

Example: Salmonella typing



Prager et al. (2003)

Principal of Polymerase Chane Reaction (PCR)

⇢Primers

- The PCR primers are artificially synthesized oligonucleotide sequences of DNA ranging from 18 to 22 bases in length, short DNA sequences which anneals at the single-stranded template DNA at its exact complementary position.

⇢dNTPs:

- Deoxynucleotide triphosphates are artificially synthesized nucleotides which bind to the growing DNA strand. With the help of the Taq DNA polymerase, the dATP, dGTP, dCTP and dTTP binds at its complementary nucleotides on the growing DNA strand.

⇢Taq DNA polymerase:

- The Taq DNA polymerase settles at the ssDNA- primer junction and utilizes it as a substrate for the catalytic reaction.

LOHMANN
BREEDERS

Types of PCR (1)

⇢Conventional PCR:

- Utilizes only a simple Taq DNA polymerase and no modifications.

➢Gradient PCR:

- For optimizing the PCR reaction, different temperature gradients are created in a machine.

➢Realtime RT PCR:

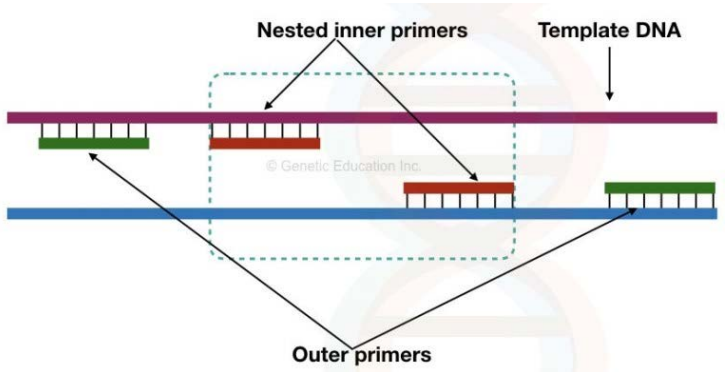
- A probe attached with a fluorochrome emits fluorescence once it is hydrolyzed from the template and the template is measured. The amount of fluorescence emitted is directly proportional to the amount of DNA present in the sample.

➢Reverse transcription PCR:

- An enzyme called reverse transcriptase converts the total mRNA into the cDNA which is measured using the same chemistry of the real-time PCR.

LOHMANN BREEDERS

Types of PCR (2)



➢ Multiplex PCR:

- Multiplex PCR amplifies multiple DNA template regions simultaneous using a different set of primers in a single PCR reaction.

➢ Nested PCR:

- The first set of primer binds outside of our target DNA and amplifies larger fragment, this set of primer is referred to as an outer primer. Another set of primer binds specifically at the target site and in the second round of amplification, it amplifies only the target DNA, this set of primer is referred to as an inner primer.

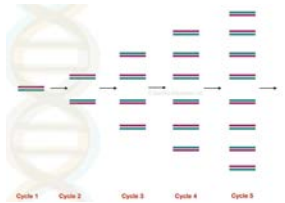
Source: https://geneticeducation.co.

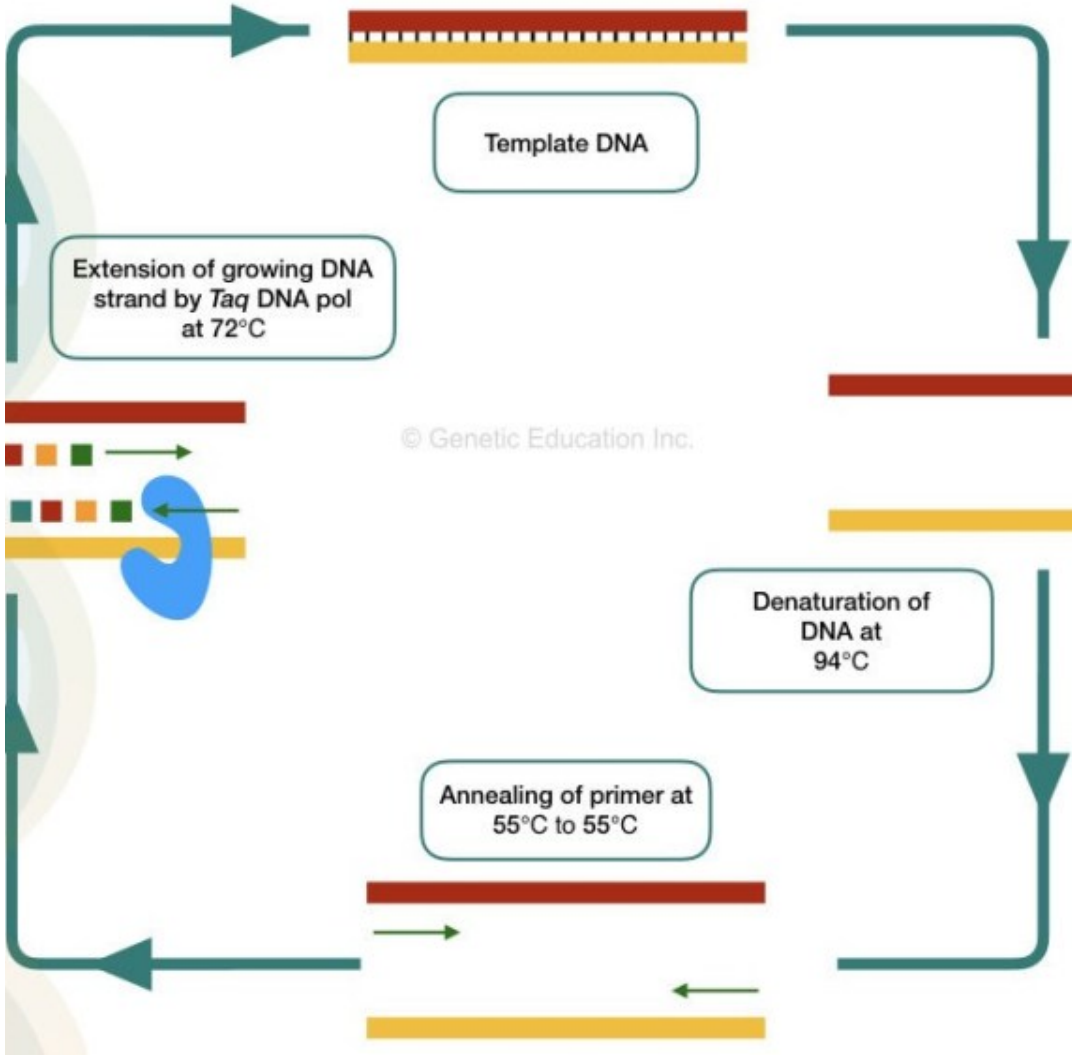
LOHMANN
BREEDERS

Principal of Polymerase Chane Reaction (PCR)

⇢PCR definition:

- Laboratory technique used to obtain multiple copies of target DNA fragments using Taq DNA polymerase in a temperature-dependent reaction (Taq: named from *Thermus aquaticus*, a thermostable bacterium)

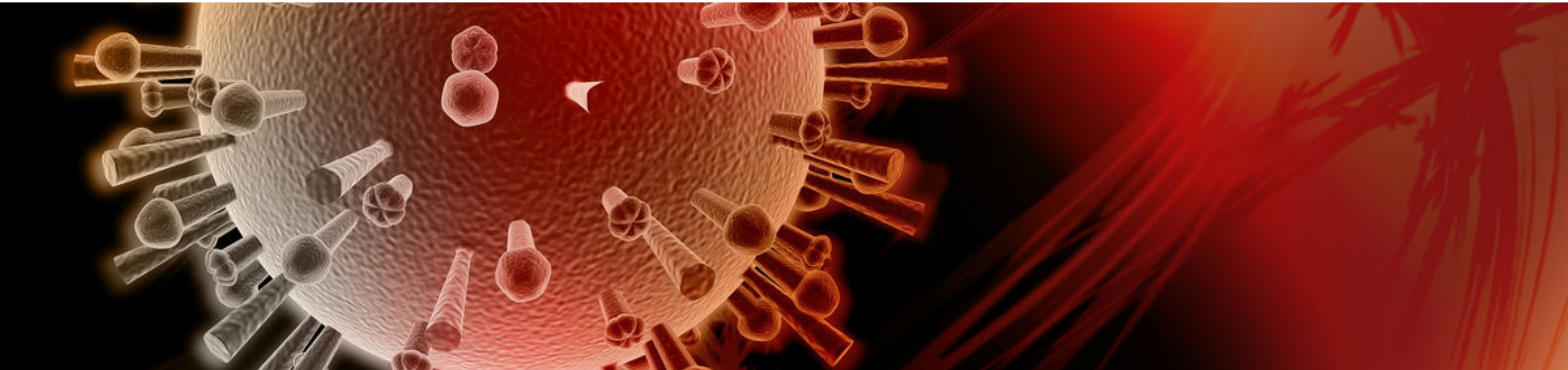




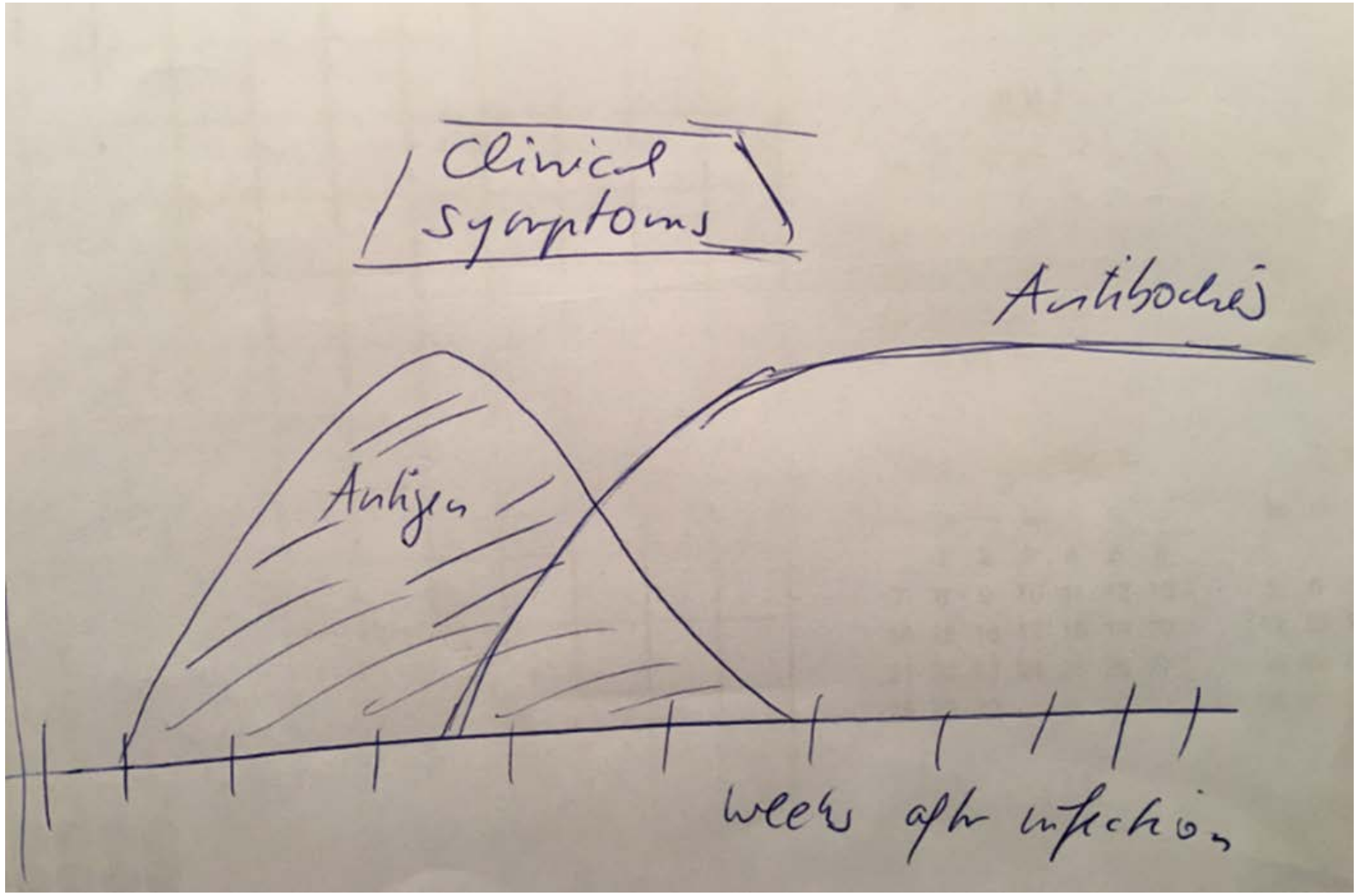
Source: https://geneticeducation.co.

LOHMANN
BREEDERS

When to test for what?

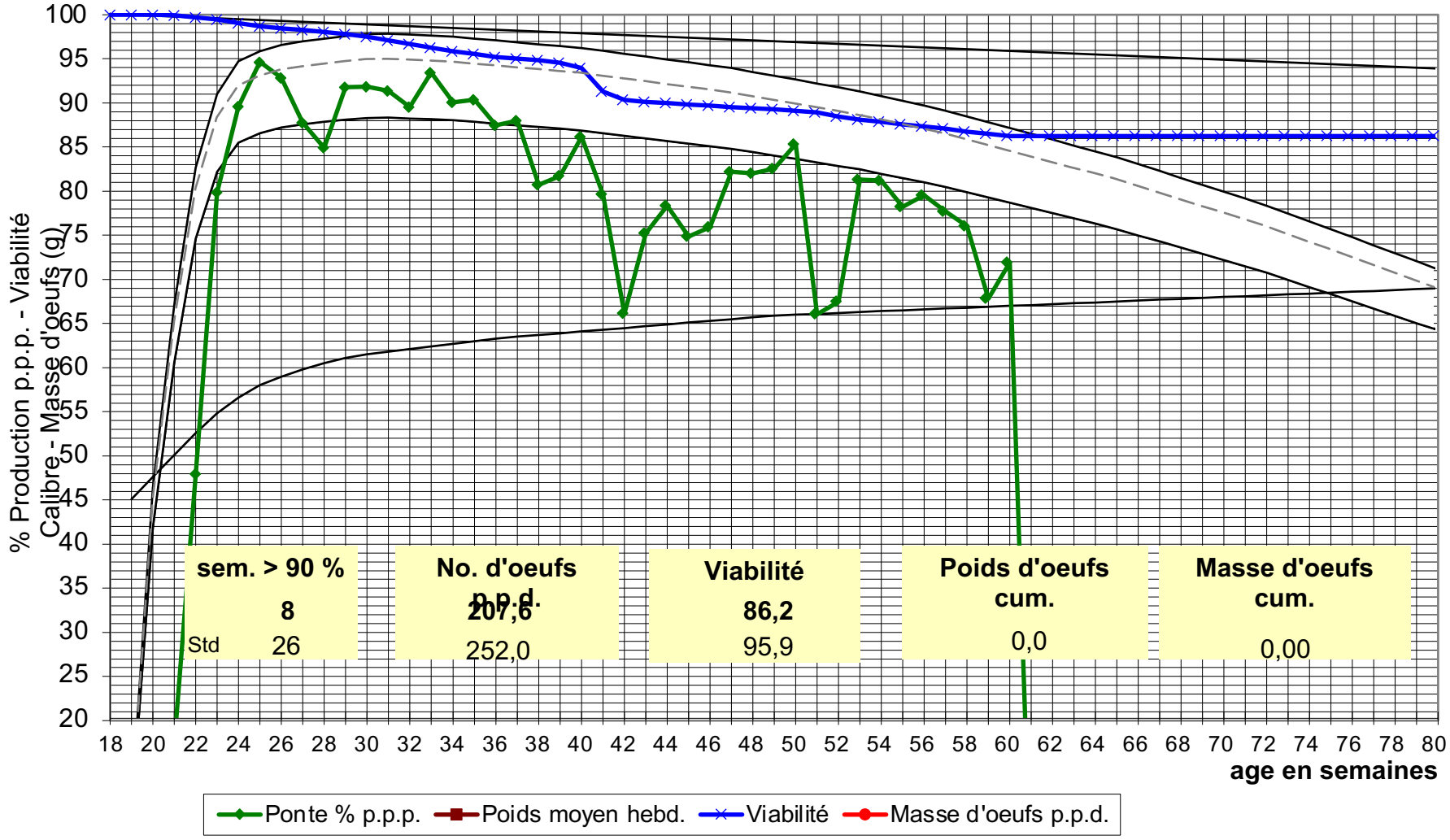


When to take which samples?

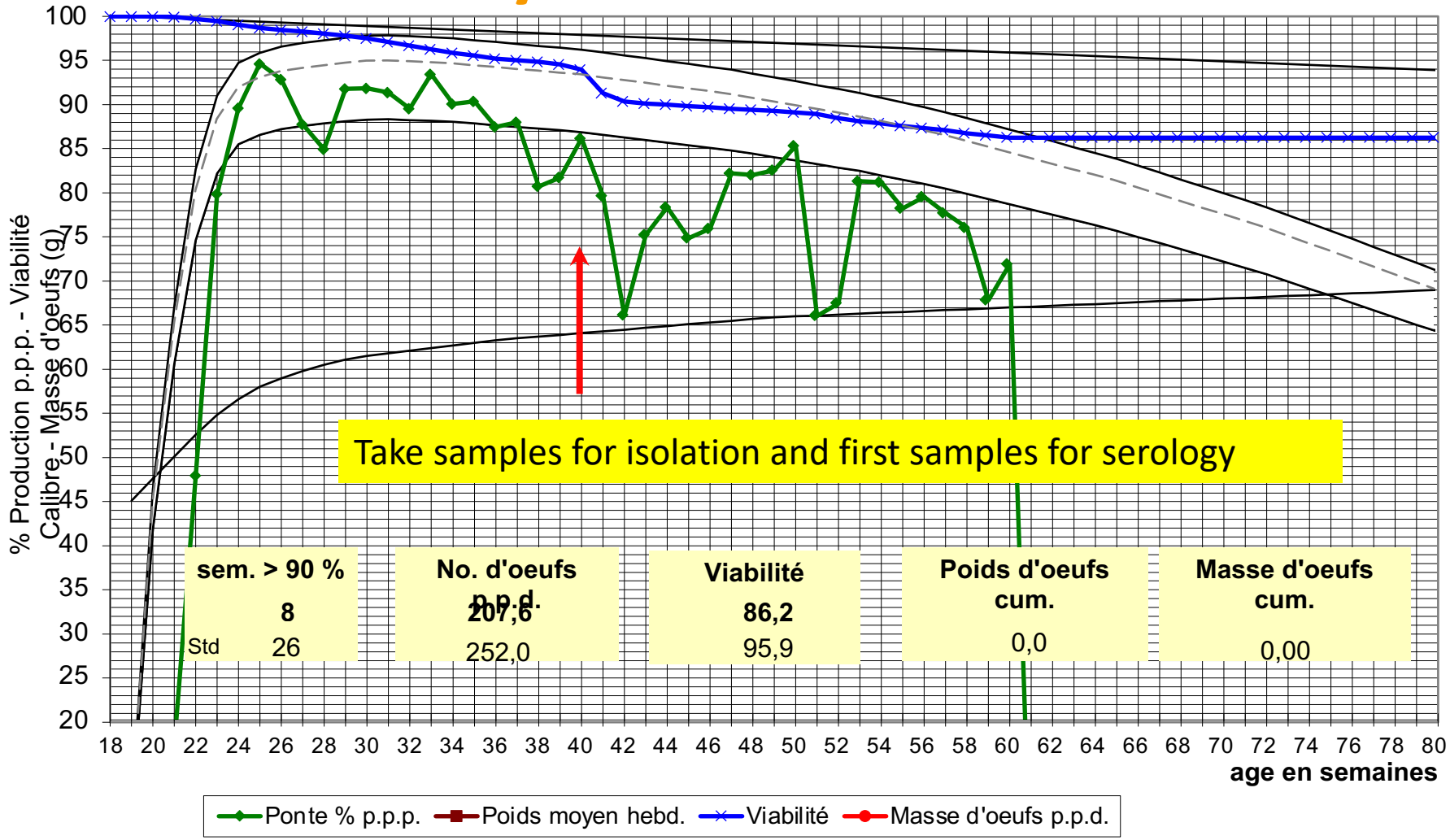


A. Bolte, 2019

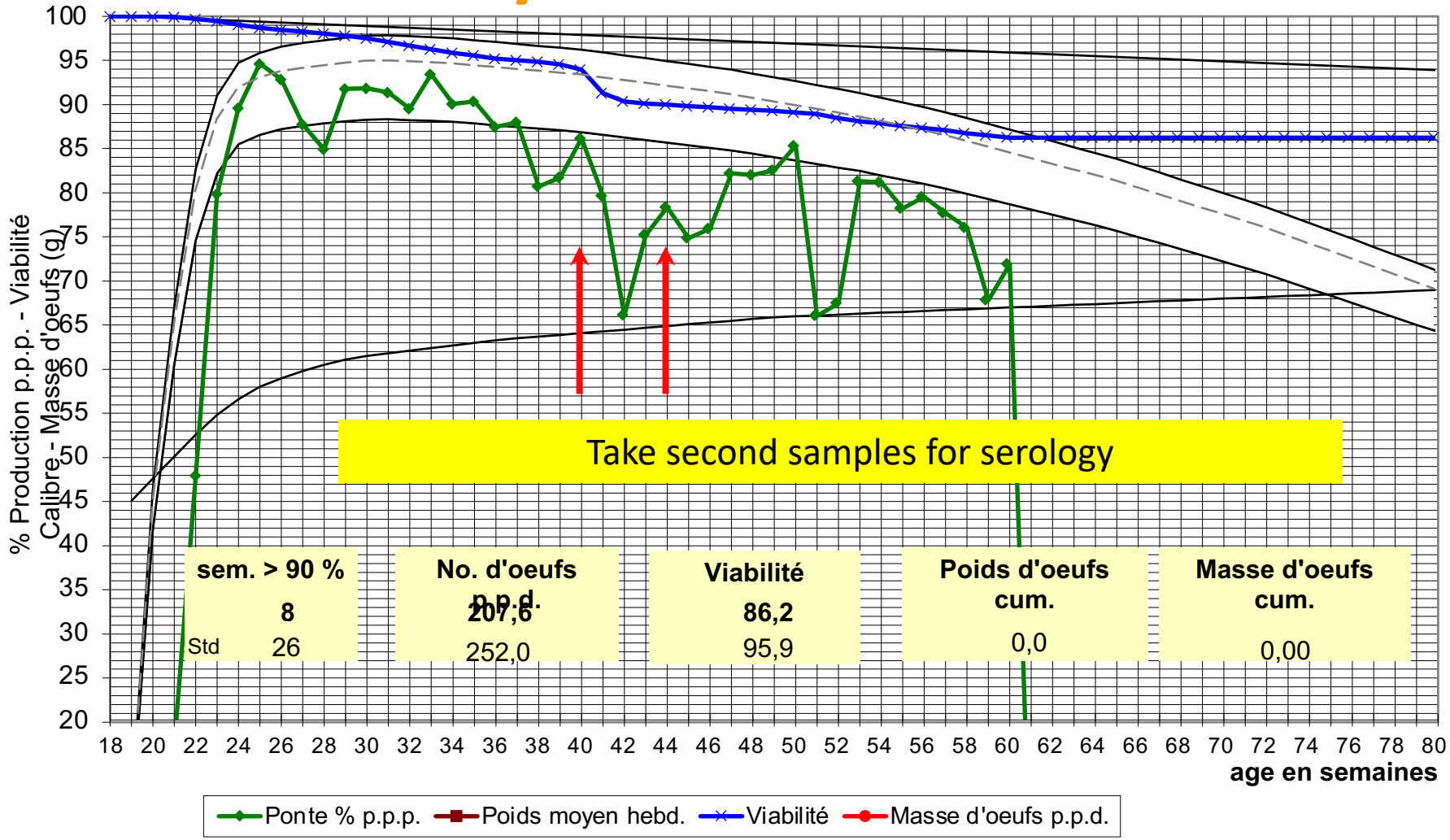
Permanent monitoring needed



At week 40 severe drop in egg production as well as increased mortality



At week 40 severe drop in egg production as well as increased mortality



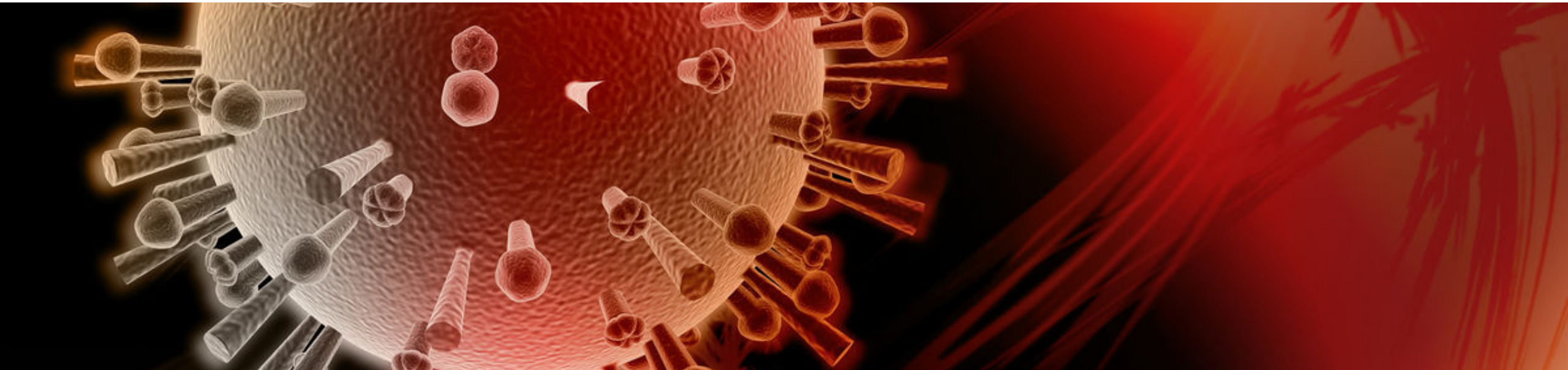
LOHMANN BREEDERS

Quick reference for sampling

	Skin lesions	Feather follicles	Cloacal swabs	Brain	Trachea/tracheal swabs	Lungs	Liver	Spleen	Pancreas	Kidneys	Oviduct	Thymus	Bone marrow	Bursa fabricius	Proventriculus/Gizzard	Caecal tonsils	various organs (tumors)	Nerves	Joints
AI			✓✓		✓✓	✓✓										✓✓			
ART					✓✓	✓✓													
IBV			✓✓		✓✓	✓✓				✓	✓					✓✓			
ND			✓✓		✓✓	✓✓										✓✓			
ILT					✓✓	✓										✓			
Pox	✓✓				✓✓														
Mycoplasma					✓✓						✓✓								✓✓
EDS			✓								✓✓								
FAdV							✓✓	✓	✓✓						✓	✓✓			
Reo			✓				✓✓	✓	✓✓							✓✓			✓✓
AE				PCR															
Gumboro (IBD)			✓											✓✓	✓				
CAV								✓✓				✓✓	✓✓						
Marek's/Leukosis		PCR													PCR			PCR	

LOHMANN
BREEDERS

Current Challenges: Pathogen identification; vaccine vs field strain and others



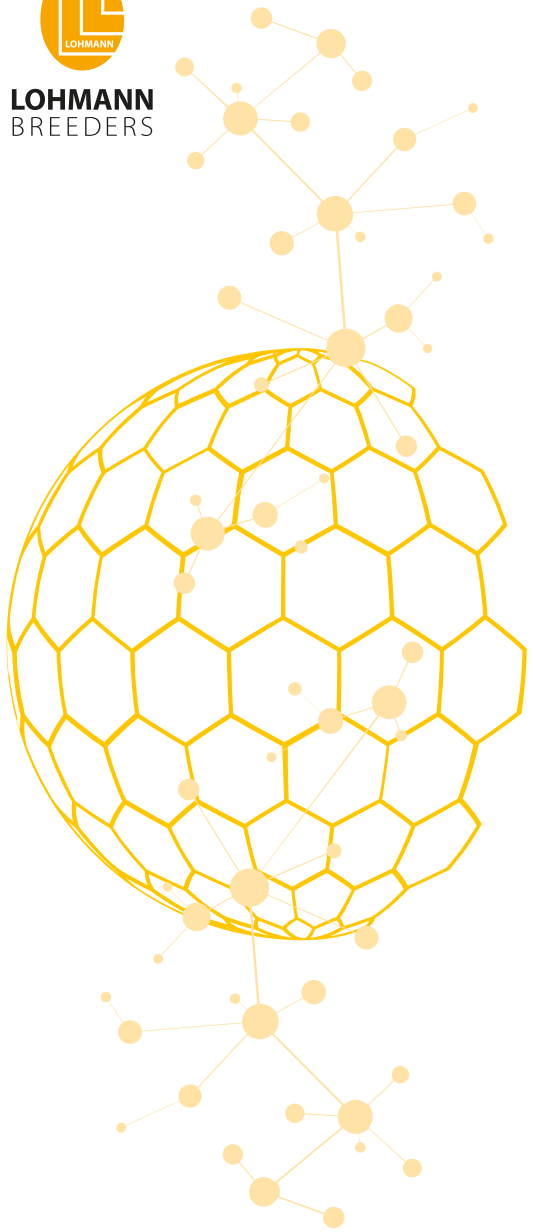
LOHMANN
BREEDERS

Pathogen identification and vaccine vs field strain: some examples for diagnostic challenges

- Mycoplasma
- Tumor diseases
- Infectious Bronchitis
- Avian Influenza

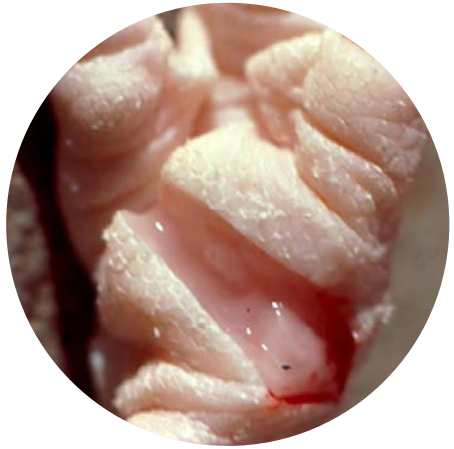
LOHMANN BREEDERS

Mycoplasma

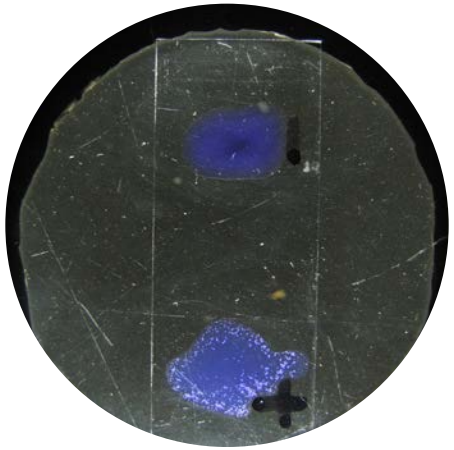


- For both Mycoplasma gallisepticum and Mycoplasma synoviae there are numerous strains in the field and there is wide variability in the relative virulence and organ tropism of these strains.
- *Mycoplasma* is spread through both vertical and horizontal transmission.
- Horizontal transmission occurs through direct contact with infected birds or indirect contact with contaminated equipment, environment, and personnel.

Diagnosis of Mycoplasma Infections



⇢Pathology



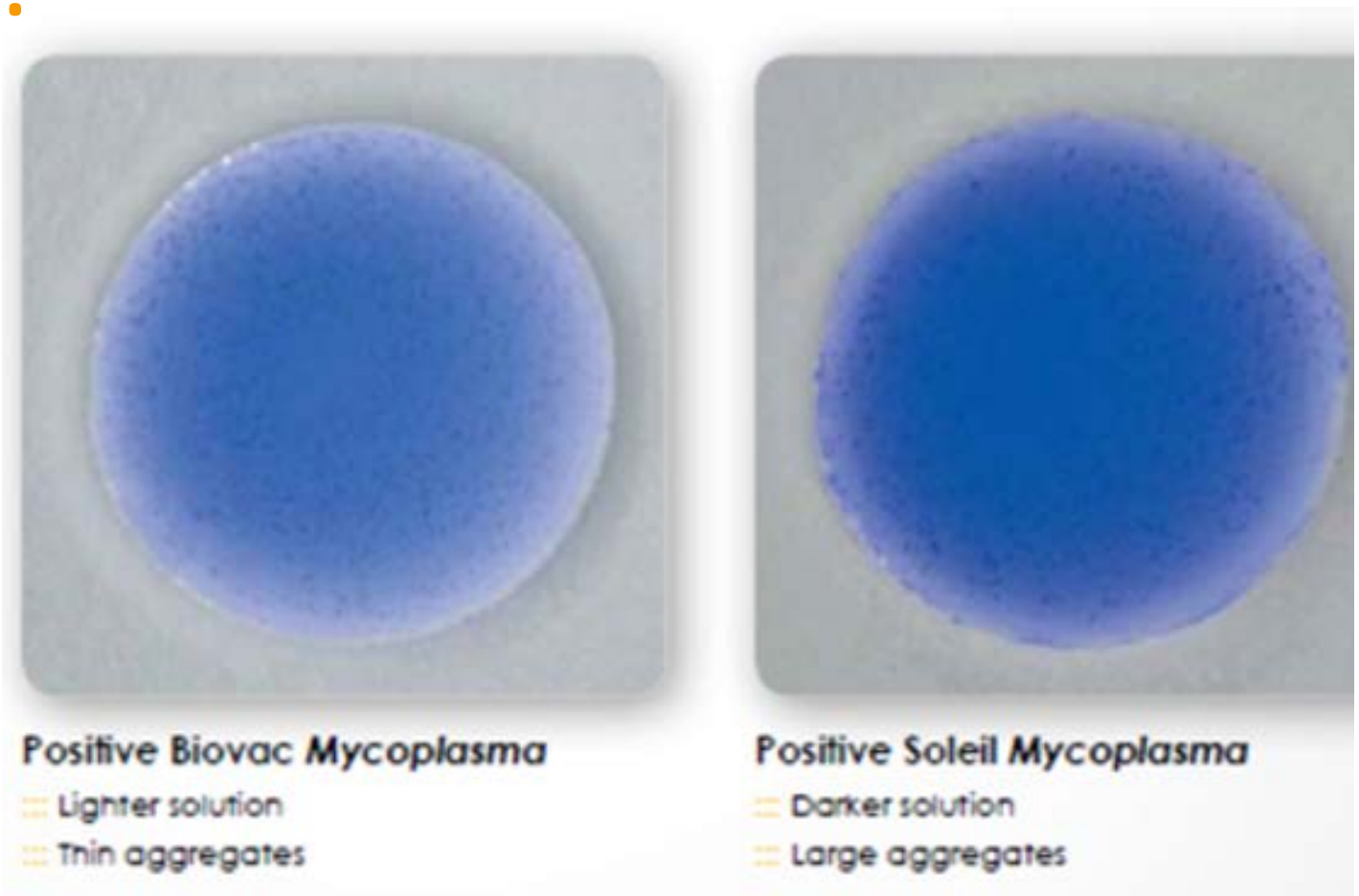
⇢Serology

⇢Isolation / Detection

LOHMANN
BREEDERS

Plate agglutination: RPA

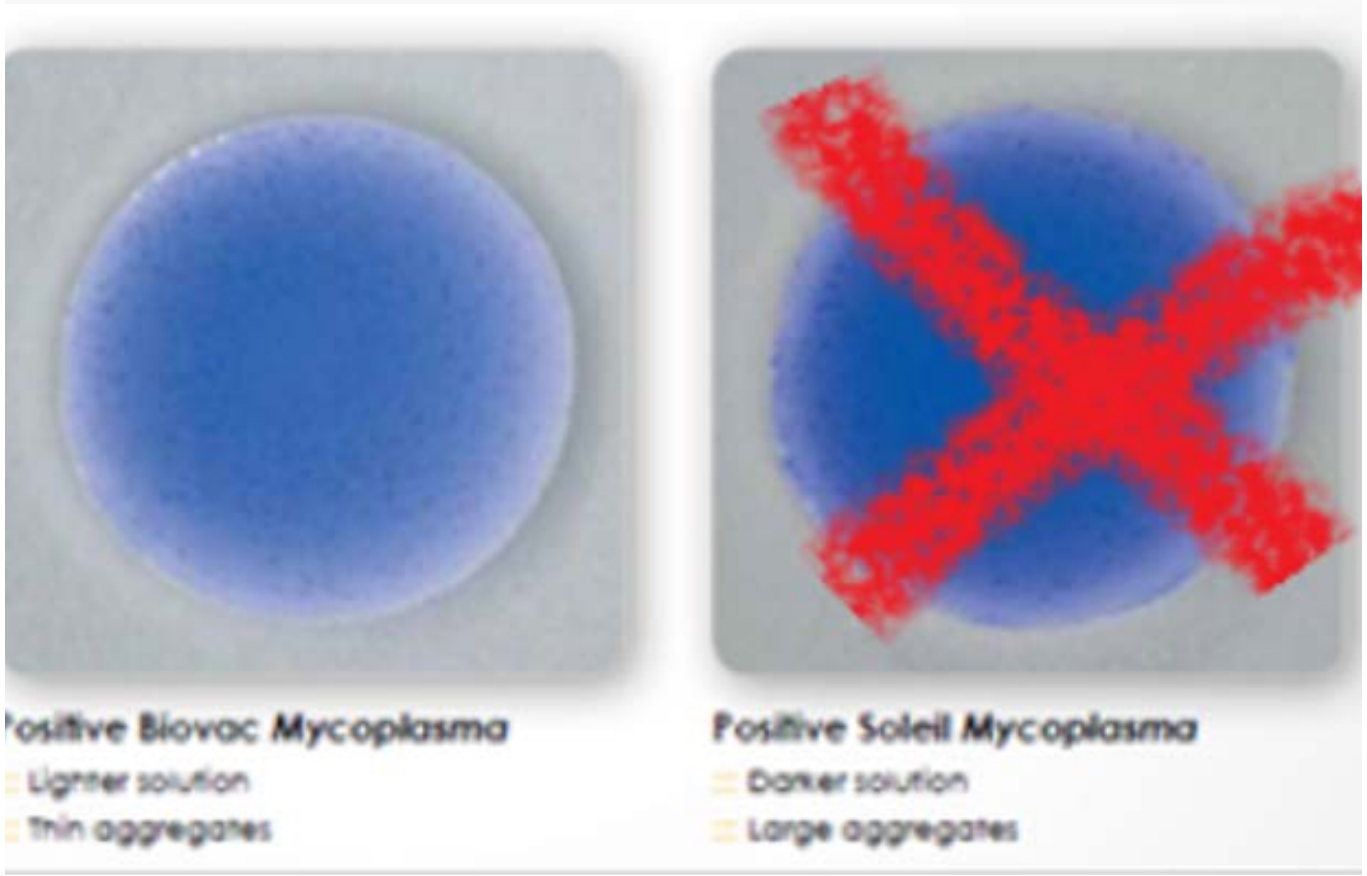
- easy and rapidly to perform(minutes)
- detects IgM and IgY
- antigen commercially available (at least for mycoplasma and salmonella)
- quality of antigens available in the market



LOHMANN
BREEDERS

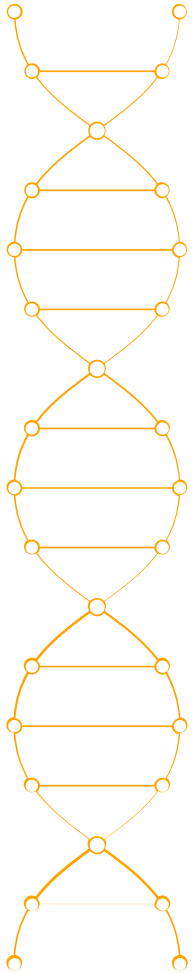
Plate agglutination: RPA

- unspecific reactions possible due to quality of serum
- false positive reactions possible after use of inactivated vaccines
- less sensitive than some of the ELISA tests (?)



LOHMANN BREEDERS

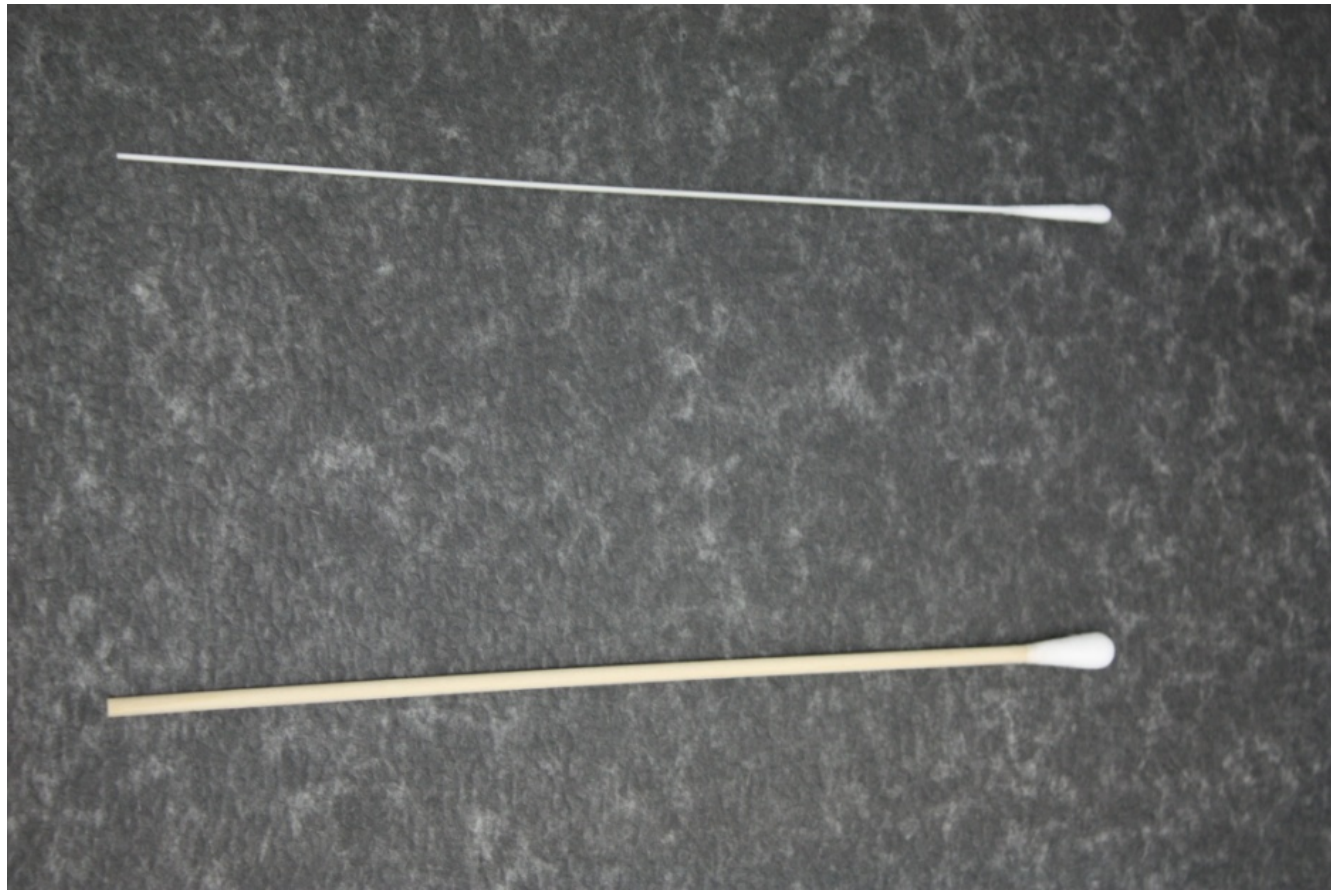
Diagnosis of Mycoplasma infections via PCR



⇢Advantages (over ELISA or culture)

- samples for the examination are easy to obtain (tracheal swabs)
- rapid diagnosis within 2-3 hours
- detection of infection short time after exposure
- high sensitivity

Diagnosis of Mycoplasma infections via PCR



- **For sampling:**

⇢ **- small swabs for trachea; pools of ten**

⇢ **-larger (wooden) swabs for cloaca; pools of five**

⇢ **thoroughly shaking out in PBS (phosphate buffered saline) solution**

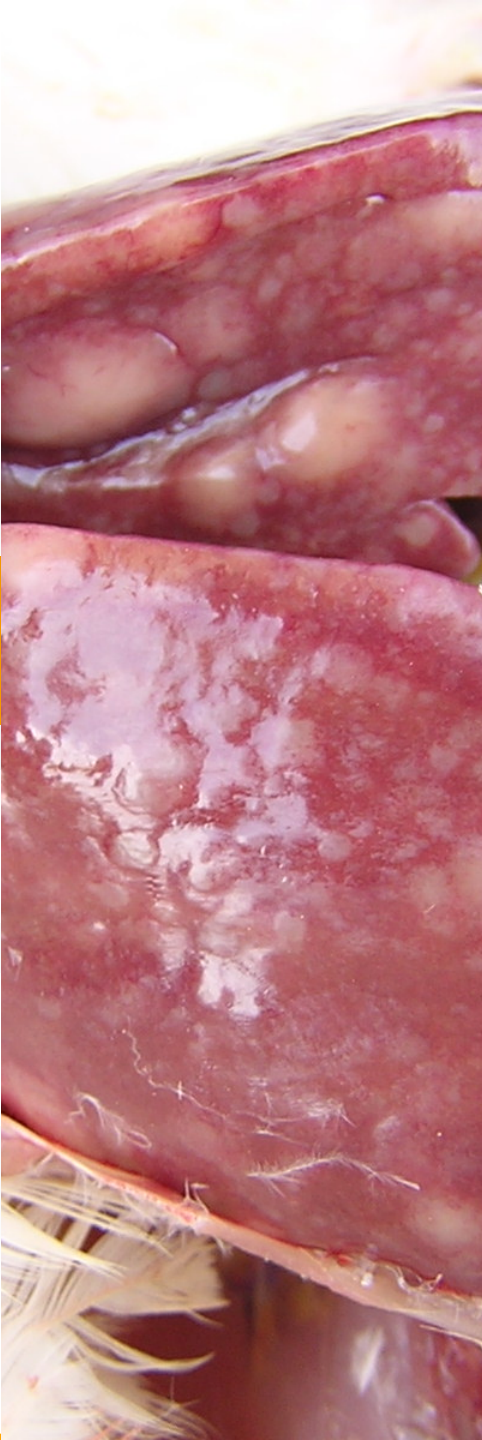
Ms field vs Ms-H: DIVA PCR

Target	Channel	Result				
Internal Control	Hex	Positive	Positive/ negative	Positive/ negative	Positive/ negative	Negative
MS field	FAM	Negative	Positive	Negative	Positive	Negative
MS-H	Cy5	Negative	Negative	Positive	Positive	Negative
Result: Ms field		No	Yes	No	Yes	Invalid
Result: Ms-H		No	No	Yes	Yes	invalid

LOHMANN
BREEDERS

Pathogen identification and vaccine vs field strain: some examples for diagnostic challenges

- Mycoplasma
- Tumor diseases
- Infectious Bronchitis
- Avian Influenza

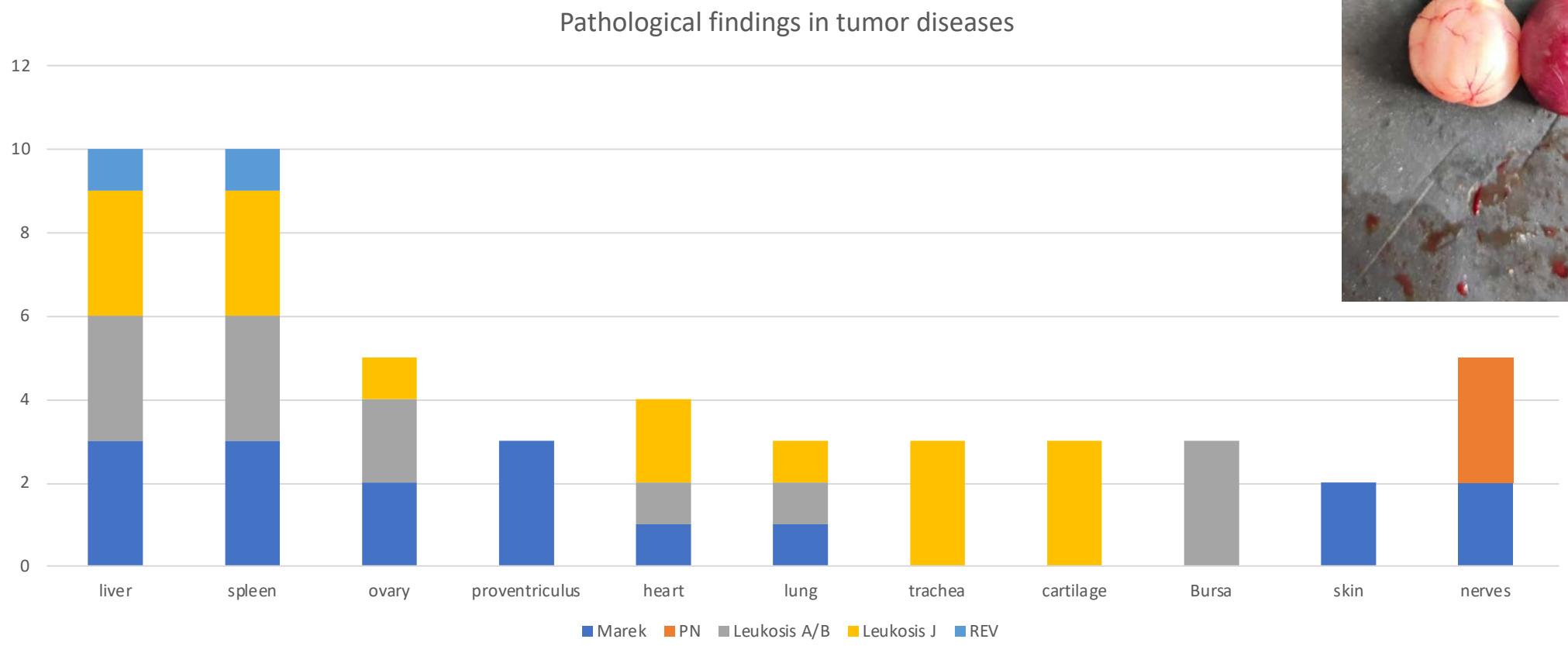


LOHMANN
BREEDERS

Marek‘s versus Avian Leukosis

	Marek	ALV A/B
occurence	world-wide	sporadic
prevalence	high	eradicated from primary breeder populations
age	> 6 weeks	> 30-35 weeks
frequency	0-50%	>2-5%
potential source	ubiquitous	back-yard, contaminated vaccines

Differential Diagnosis of Tumors



LOHMANN BREEDERS

Avian Leukosis Viruses

Subgroup A: exogenous virus chickens, common	Subgroup B: exogenous virus chickens, common	Subgroup C: exogenous virus chickens, rarely	Subgroup D: exogenous virus chickens, rarely
Subgroup E: endogenous virus chickens	Subgroup F: endogenous virus pheasants	Subgroup G: endogenous virus pheasants	Subgroup H: endogenous virus partridge
	Subgroup I: endogenous virus quail	Subgroup J: exogenous virus meat-type chickens	

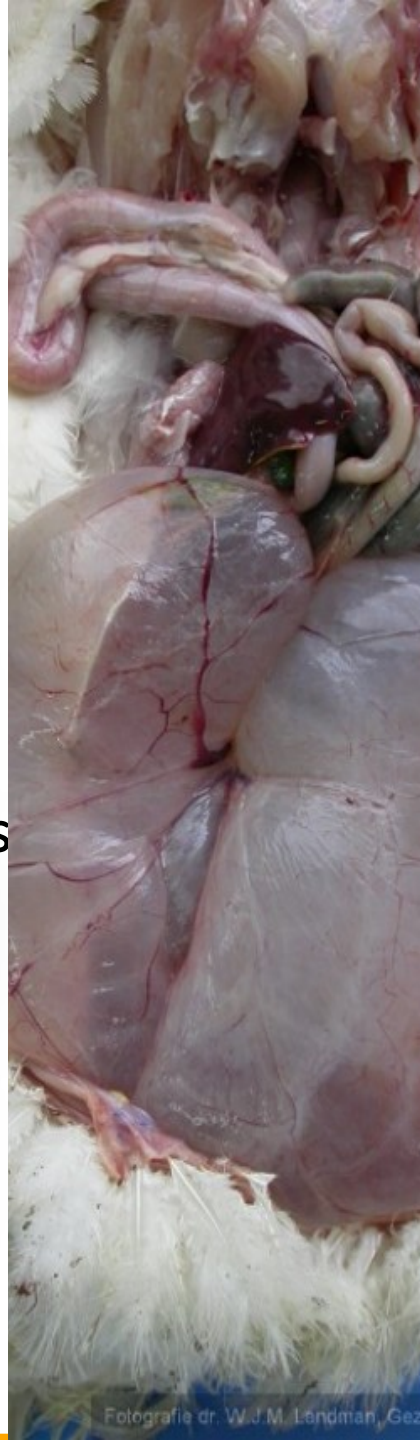
Avian Leukosis Virus (ALV): Diagnosis

Detection of	Test System	Subgroups	Sample	Remarks
antibodies	ELISA IDEXX	A + B (?)	serum	screening for infection
	ELISA J	J	serum	screening for infection
	SN	all subgroups	serum	screening for infection
antigen	ELISA IDEXX	p 27, all subgroups	cloacal swabs, meconium, albumen, (serum)	eradication
	virus isolation C/O	all subgroups	serum	detection of infectious virus
	virus isolation C/E	A - D, J	serum	diff. endog./exog. virus
	PCR testing	all subgroups, depending on primers	various	

LOHMANN
BREEDERS

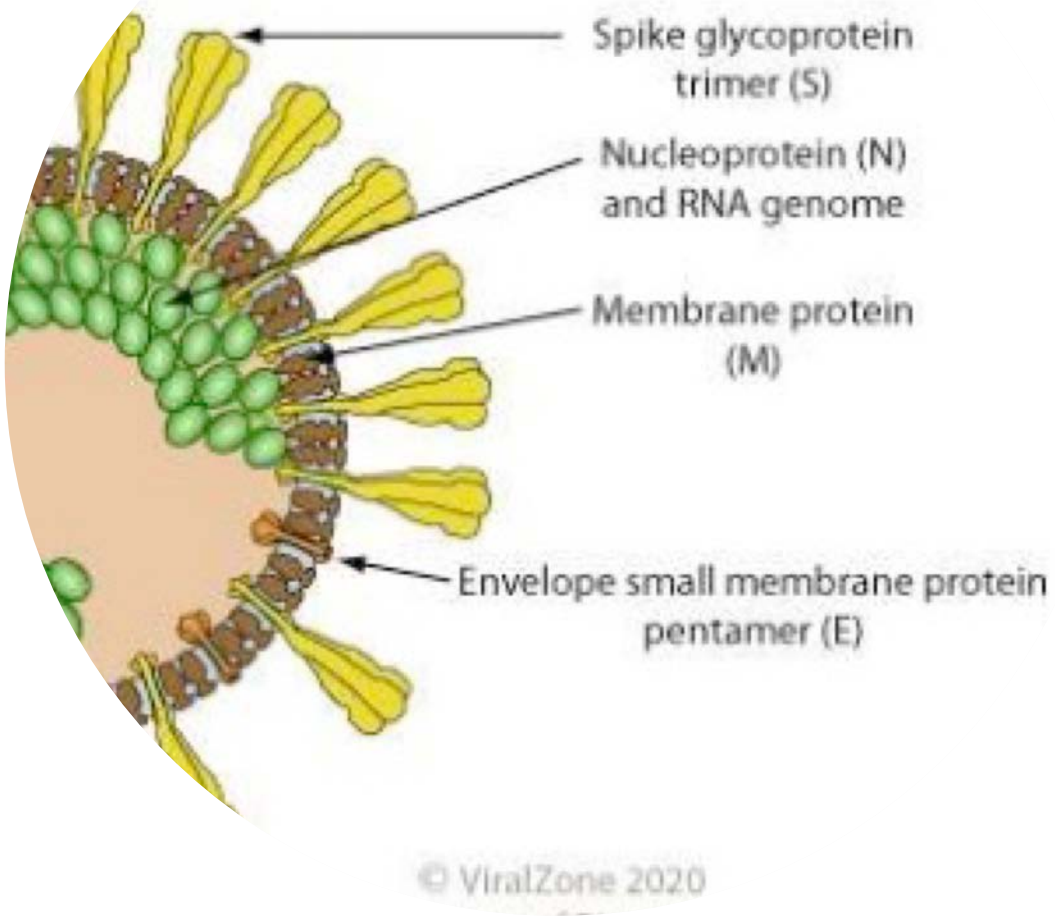
Pathogen identification and vaccine vs field strain: some examples for diagnostic challenges

- Mycoplasma
- Tumor diseases
- **Infectious Bronchitis**
- Avian Influenza



LOHMANN
BREEDERS

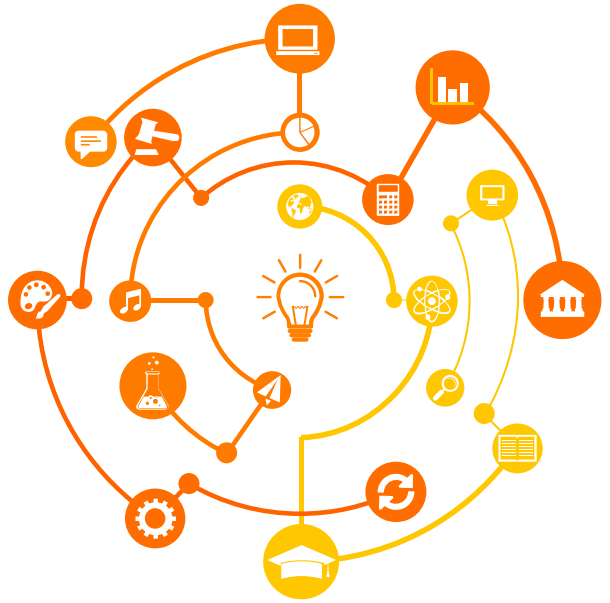
Avian Infectious Bronchitis



- enveloped single stranded RNA virus
- high incidence of mutations in the genome
- S1 spike protein determines different serotypes and induces HI and neutralising antibodies

Infectious Bronchitis: Serology

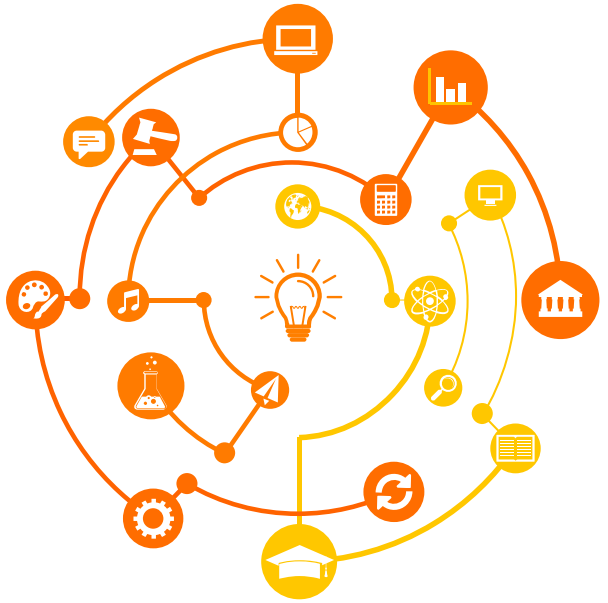
- **Group-specific**
  - **Agargel Precipitation test (AGPT)**
  - **ELISA**
- **Serotype-specific**
- **Haemagglutination Inhibition (HI) Test**
- **Virus Neutralization Test using:**
  - **Chicken Embryo Kidney Cells (CEKC)**
  - **Tracheal Organ Cultures (TOC)**

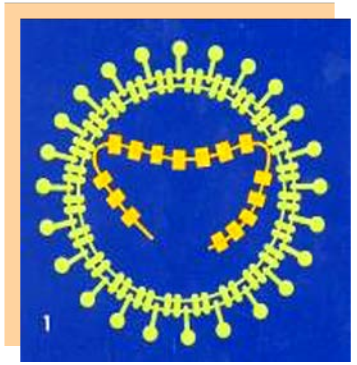


Important IB Serotypes

- Massachusetts 41 (1941)
- Connecticut (1956)
- Iowa 97 /609 (1958)
- H 52 (1960)
- Holte, Gray (1962)
- IBV-T (1963)
- JMK (1964)
- Florida (1971)
- Dutch variants D-274/ D-1466 (1977), D-3128 (1983)

- PL-84084 (1986)
- IBV-G (1986)
- 793B oder 4/91 (1991)
- 614/I (1994)
- D 388 / China QX (2005)
- Variant 2 (Middle East)





LOHMANN
BREEDERS

Infectious Bronchitis:
Serology

- Agargel Precipitation test (AGPT)
- ELISA
- Hemagglutination Inhibition (HI) Test
- Virus Neutralization Test using:
    - Chicken Embryo Kidney Cells (CEKC)
    - Tracheal Organ Cultures (TOC)

LOHMANN
BREEDERS

Infectious Bronchitis: Serology vs. virus identification

- Serology for Infectious Bronchitis can give only an indication about the field virus involved in clinical or production problems as we test only for the strains, we have available as antigens
- With the now available molecular-biological methods (PCR) it is much more accurate to identify the type of viruses in the field

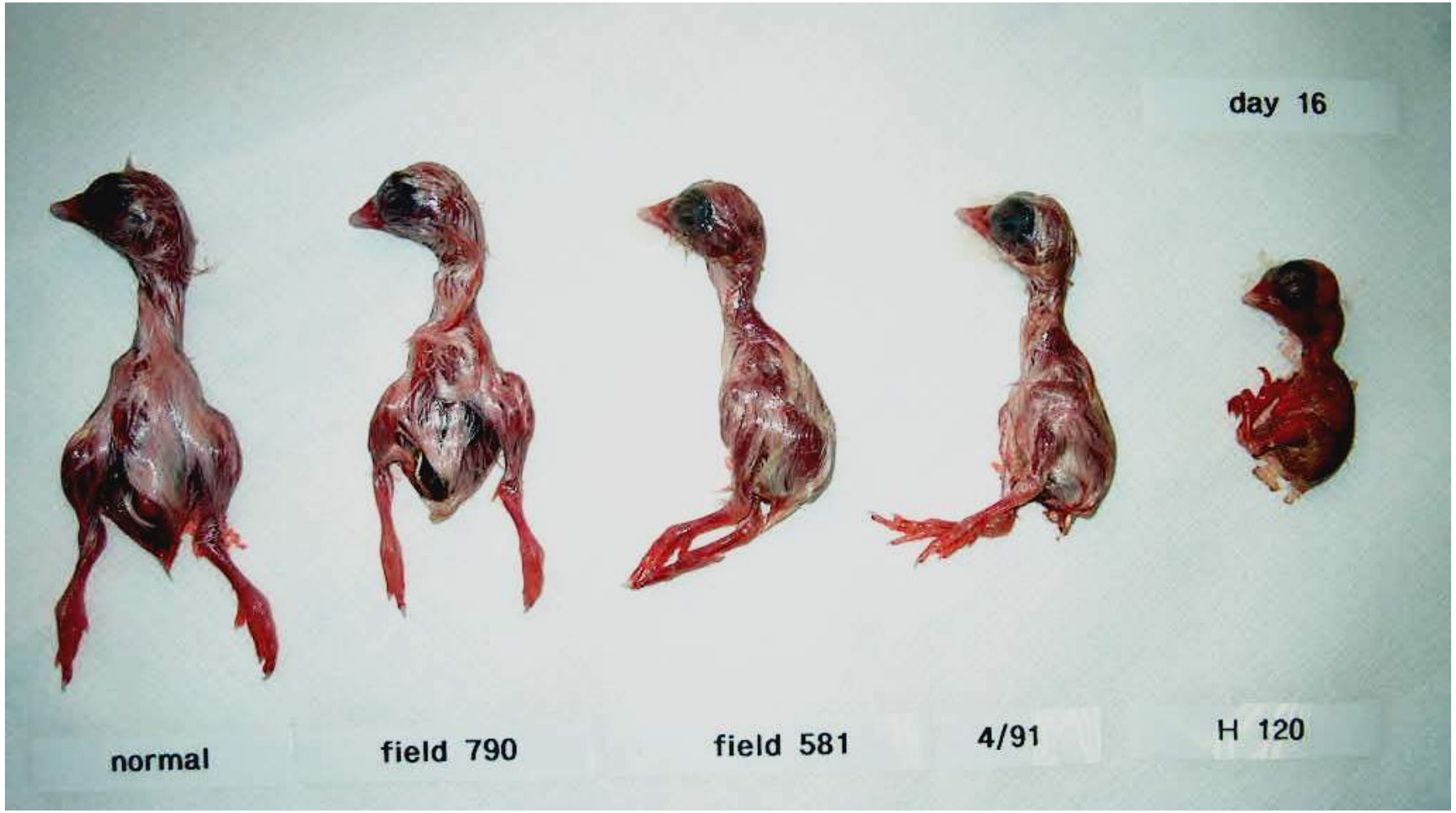
Infectious Bronchitis: Virology

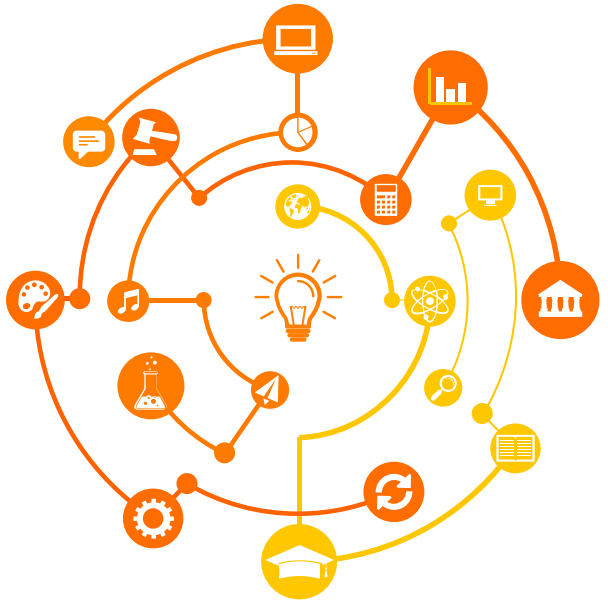
- Suitable samples for the isolation of IB are:
  - Trachea or tracheal swabs (in case of respiratory symptoms)
  - Caecal tonsils
  - Cloacal swabs

- SPF embryo culture:
  - inoculate 9-10 days old embryos into the allantoic cavity and incubate at 37°C
  - discard eggs dying within 24 hours after inoculation
  - observe embryo mortality over 7 days
  - make at least 3 passages
  - Check for specific embryo changes (dwarfing)

Infectious Bronchitis:

Virology: Embryo Adaptation



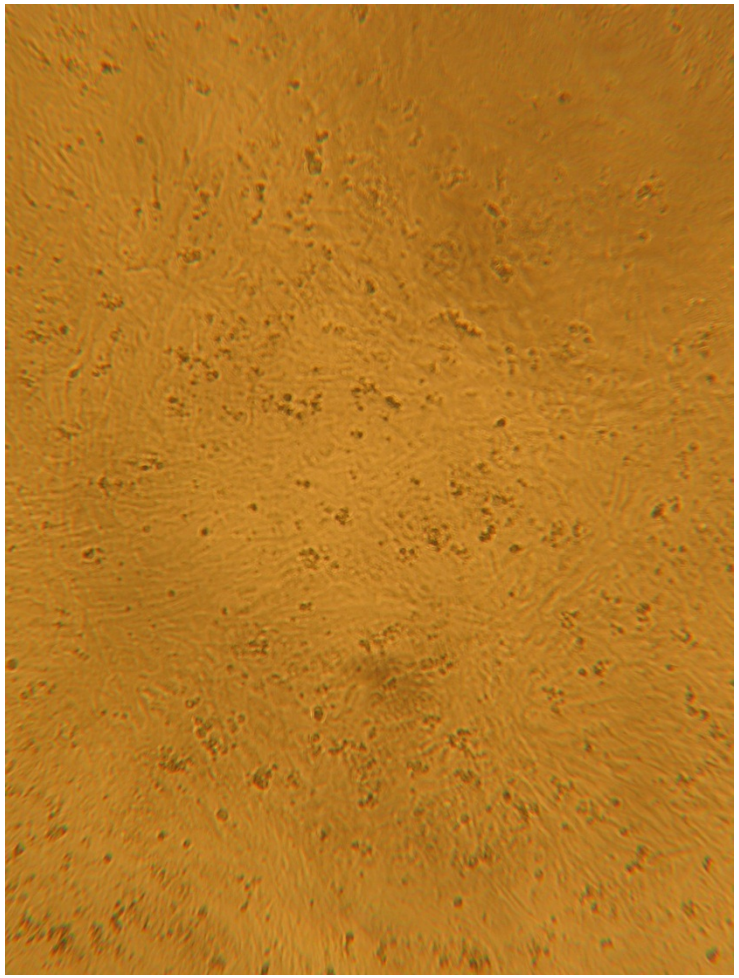


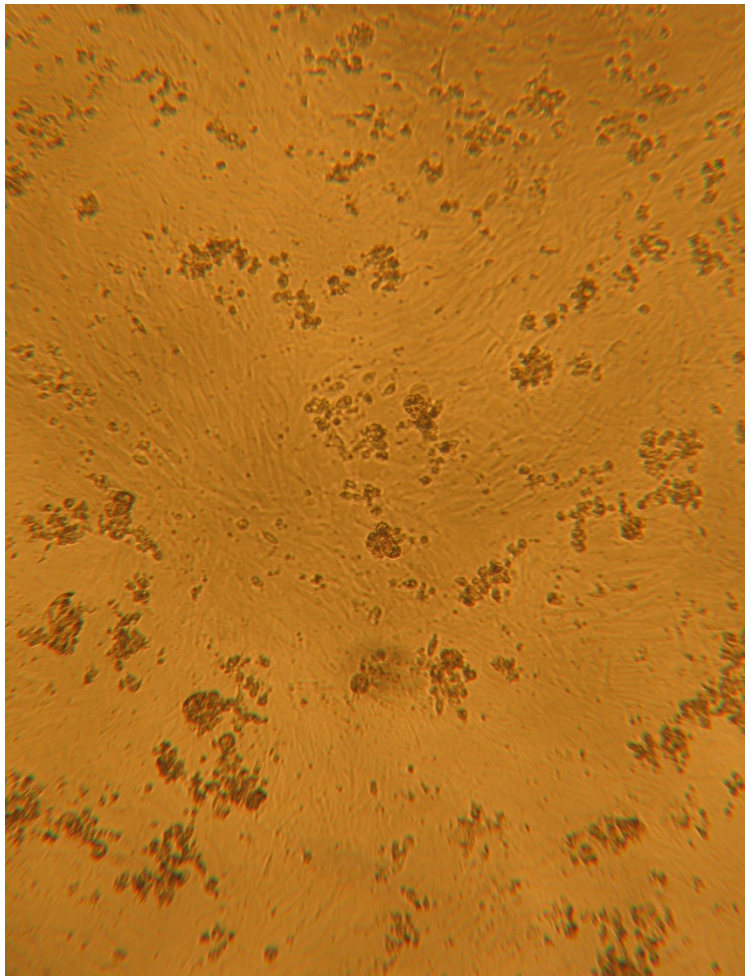
Infectious Bronchitis:

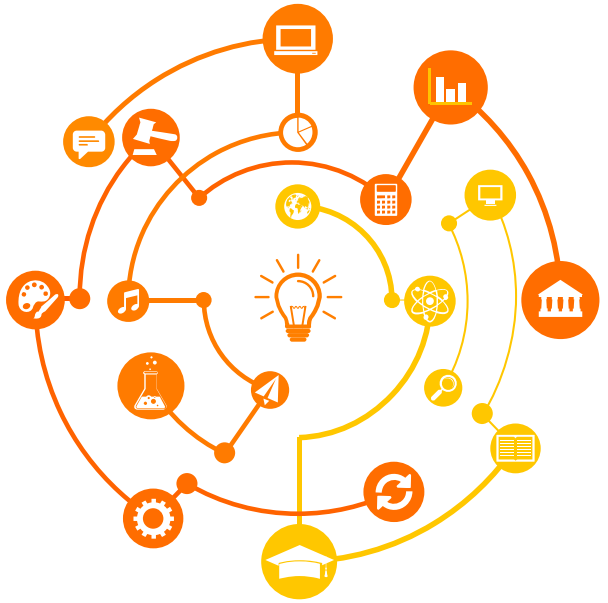
Virology: CPE in CEK cells

negative,

72 h p.i.







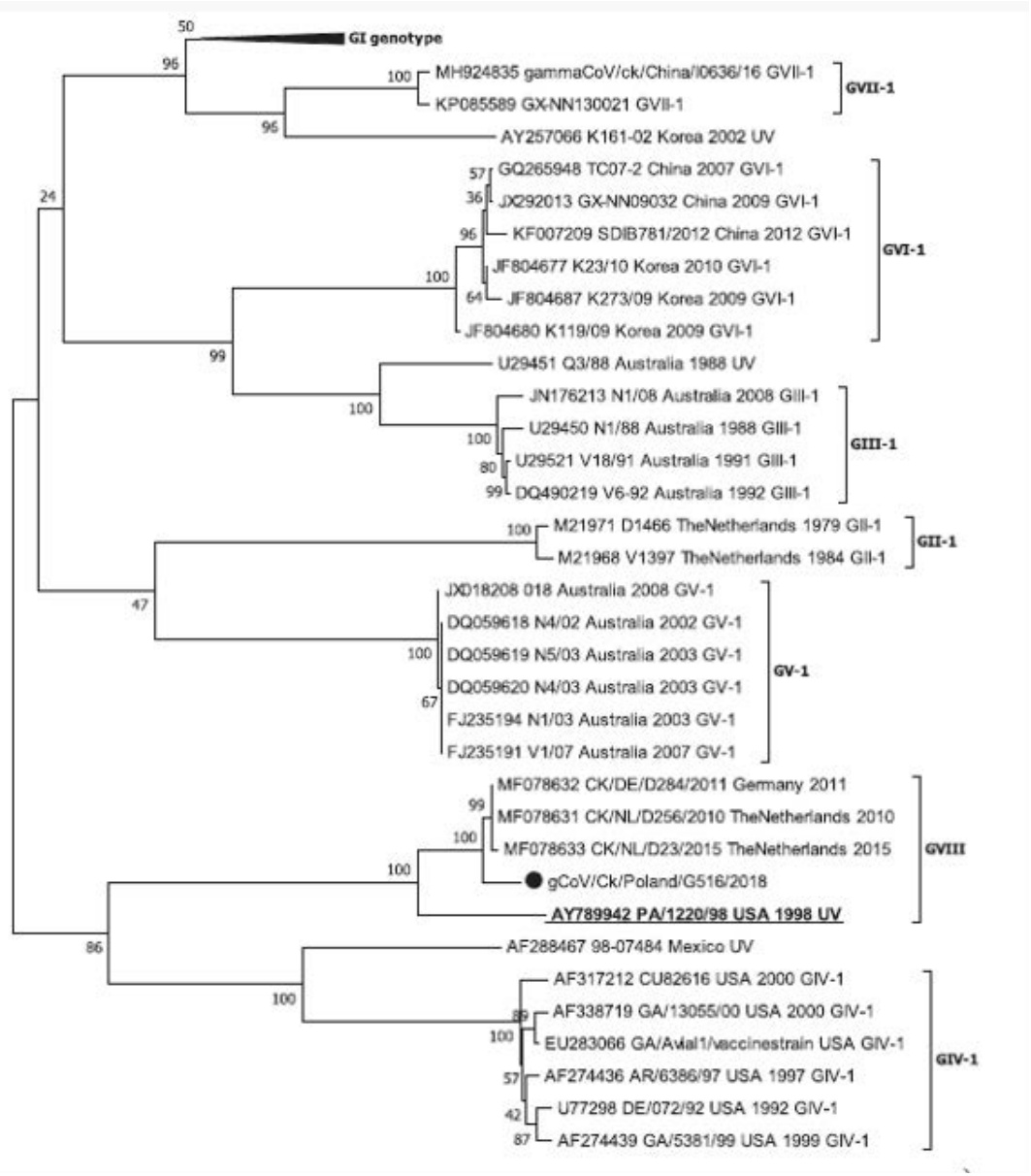
adapted strain,

72 h p.i.

LOHMANN
BREEDERS

Infectious Bronchitis

- **Phylogenetic tree based on a partial S1 coding region fragment (from 754 to 1133 nt according to the same strain sequence).**



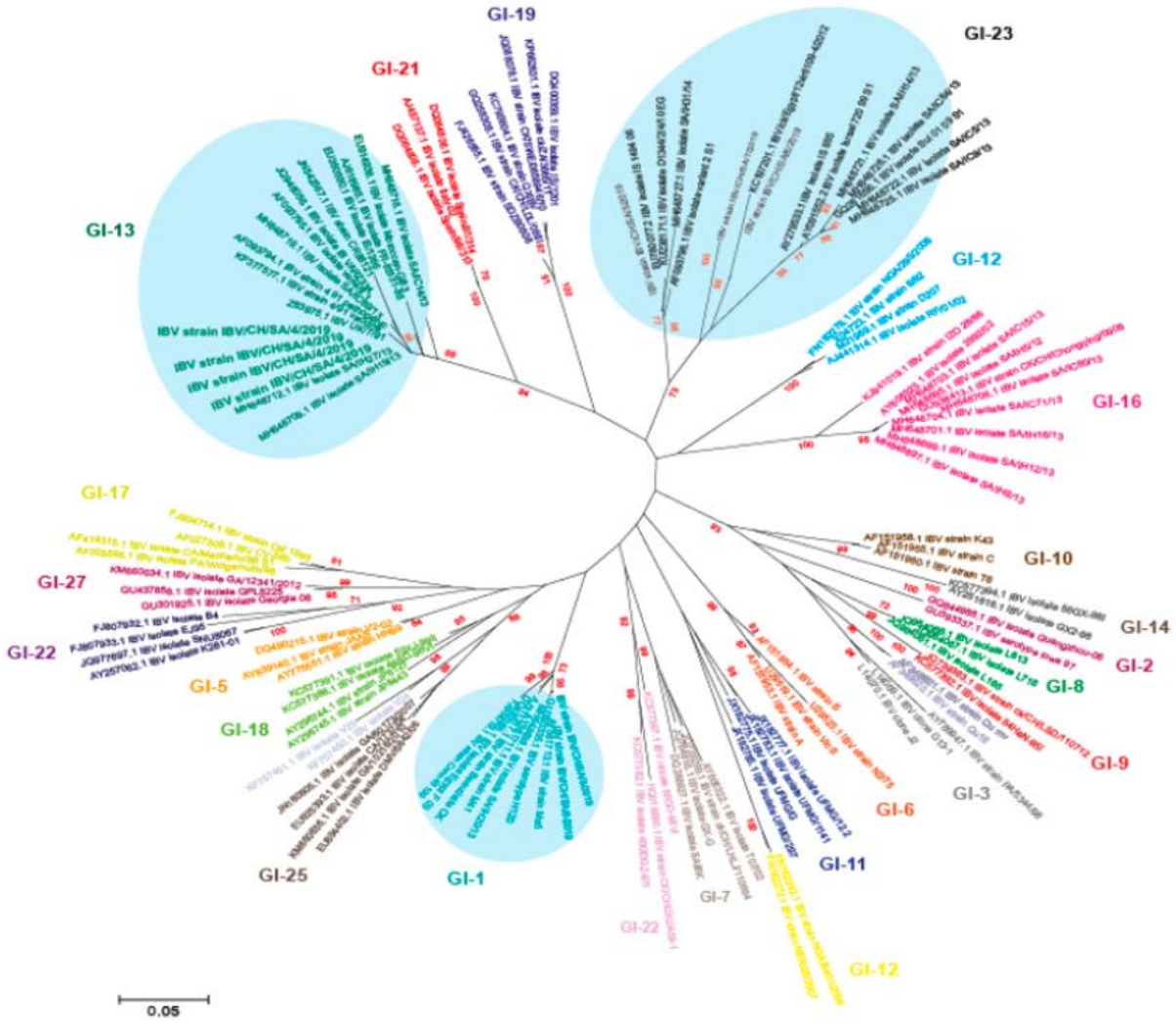
Domanska-Blicharz, K. et al; Av Path 2021 (49)

IBV live vaccines: Europe and Middle East

Product	Producer	Mass	D-274	4/91 (793B)	China-QX	Variant 2
Gallivac IB88 neo	Boehringer			CR88121		
Bioral H120	Boehringer	H 120				
Bioral H52	Boehringer	H 52				
HatchPak IB H120 Neo	Boehringer	H 120				
Cevac IBird	Ceva			Variant 1/96		
Cevac Mass L.	Ceva	Mass B-48				
Avipro H120	Elanco	H 120				
Avishield IB GI-13	GENERA			V-173/11		
Avishield IB H 120	GENERA	H 120				
Izovac IB H120	Izo	H 120				
Nobilis IB 4/91	MSD			4/91		
Nobilis IB H120	MSD	H 120				
Nobilis Ma5	MSD	Ma5				
Nobilis IB D274	MSD		D-274			
Nobilis IB Primo QX	MSD				D388	
TAbic® **IB Var**	Phibro			IS233		
TAbic® **IB Var** 206	Phibro					variant 2
Poulvac IB H 120	Zoetis	H 120				
Poulvac IB Primer	Zoetis	H 120	D-274			
Poulvac IB QX	Zoetis				L1148	

Product and producers names may differ depending on registration documents

LOHMANN
BREEDERS



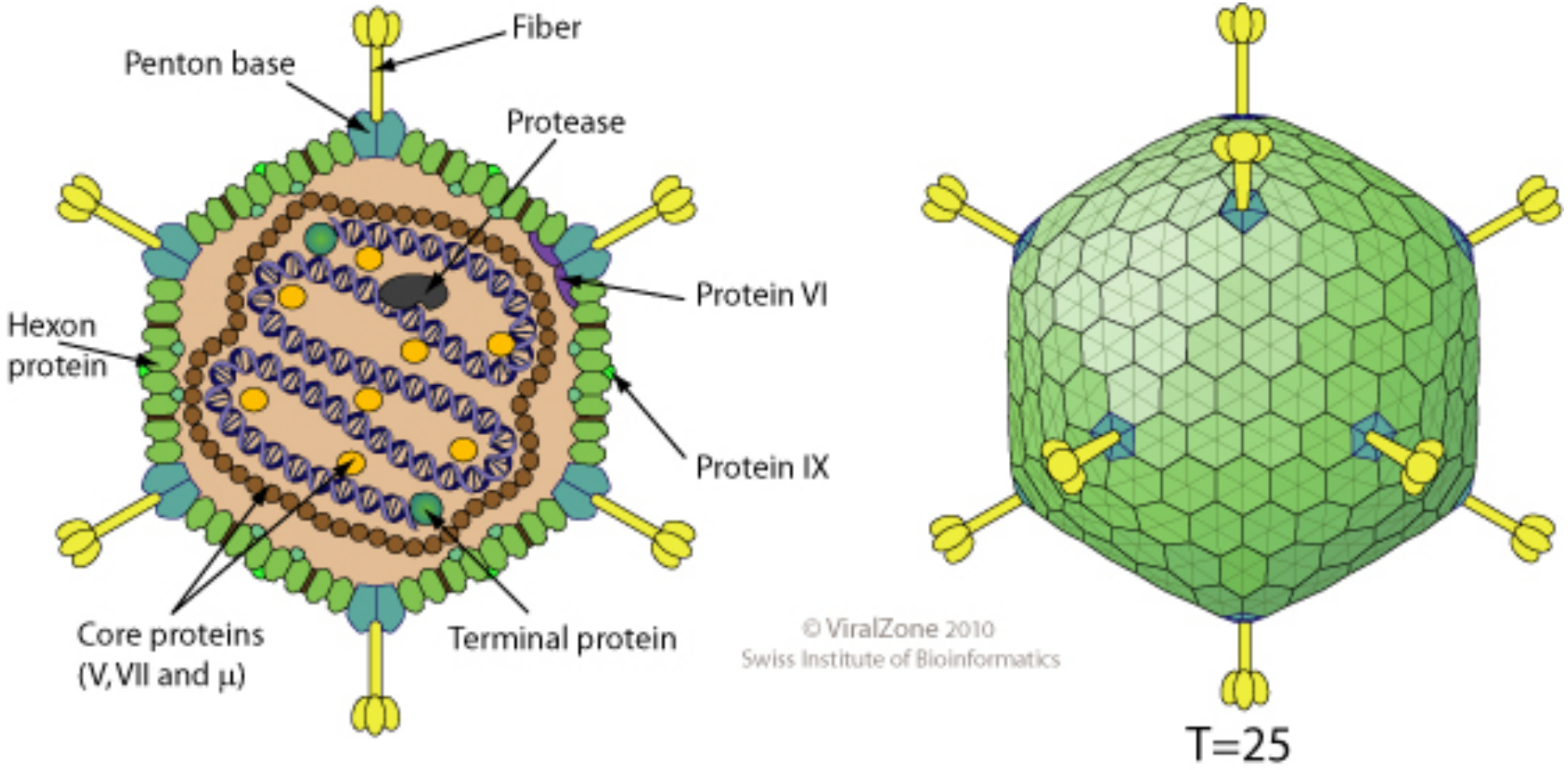
- **Phylogenetic analysis based on S1 gene of the newly identified infectious bronchitis viruses (IBVs) in Saudi Arabia during 2019 showed the clustering pattern for the studied isolates with different IBV lineages labelled with circle**

Rohaim, M. et al; Genes 2020 (11).

Properties of Adenoviruses

- Adenoviruses represent the largest nonenveloped viruses with Icosahedral structure
- Size of 70-90 nm
- The virion also has a unique "spike" or fiber associated with each penton base of the capsid that aids in attachment to the host cell
- The adenovirus genome is linear, non-segmented double-stranded (ds) DNA

Morphology of Adenoviruses



Taxonomy of Adenoviruses in Poultry

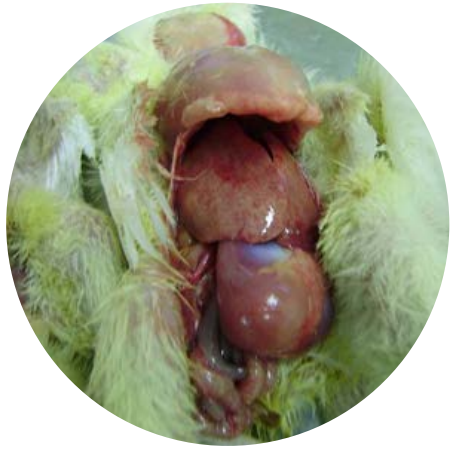
Family	Genus	Species
Adenoviridae	Aviadenovirus (Group 1 avian adenoviruses)	Fowl Adenovirus (FAdV) Species A – E Serotype 1 – 11 (ICTV)
	Siadenovirus (Group 2 avian adenoviruses)	• Hemorrhagic Enteritis of Turkeys (HEV) • Marble Spleen Disease of Pheasants • Avian adenovirus splenomegaly (AAS) in chickens
	Atadenovirus (Group 3 avian adenoviruses)	Duck Adenovirus 1 (DAdV-1; Egg Drop Syndrome, EDS)

LOHMANN BREEDERS

Aviadenovirus Classification (FAdV)

Species	ICTV	EU	US	Strains
A	1	1	1	Celo, 112,QBV, H1
B	5	5	8	TR22, M2 Tiptron, IBH-2A
C	4	4	4	KR5, 506, H2, K31, 61, J2-A
	10	11	10	C2B, M11, CFA20, SA2, C-2B
D	2	2	2	SR48, 685, H3, P7-A, GA1-1, Z7
	3	3	3	SR49, 74, H5, 75-1A1
	9	10	9	A02, 90, CFA19, A2-A
	11	12	12	UF71, 380
E	6	6	5	CR119, 168
	7	7	11	YR36, X11, X11-A, 122
	8a	8	6	TR59, 58, CFA40, T8-8
	8b	9	7	764, VRI-33, B-3A

Disease Associated with FAdV Infections



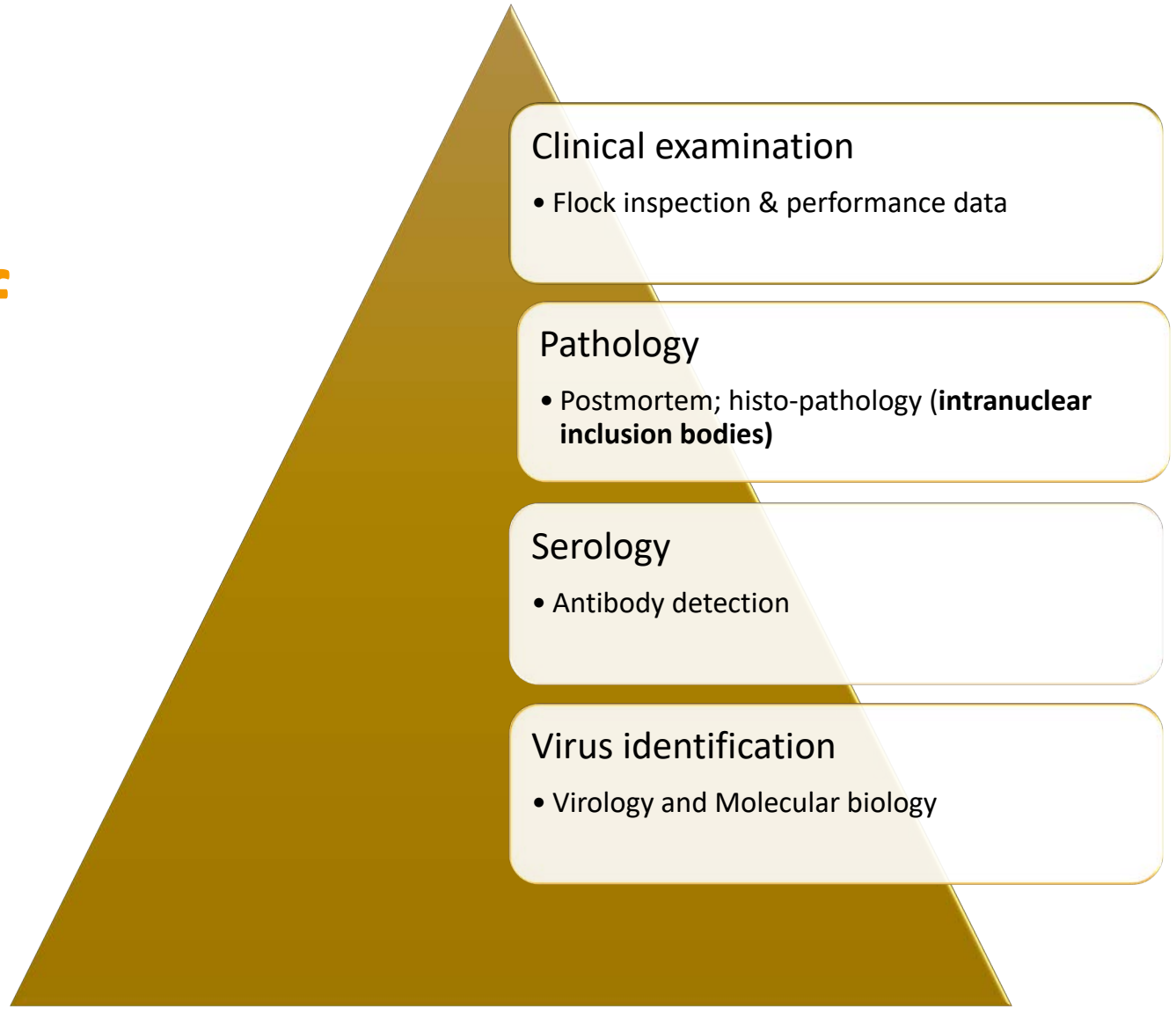
❖ Inclusion Body Hepatitis

⇢ Hydropericardium Syndrome

⇢ Gizzard erosion

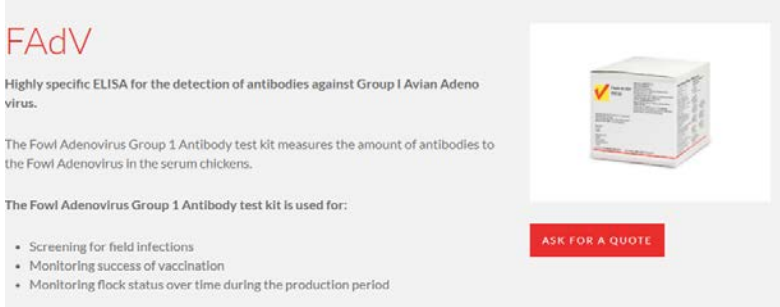
LOHMANN
BREEDERS

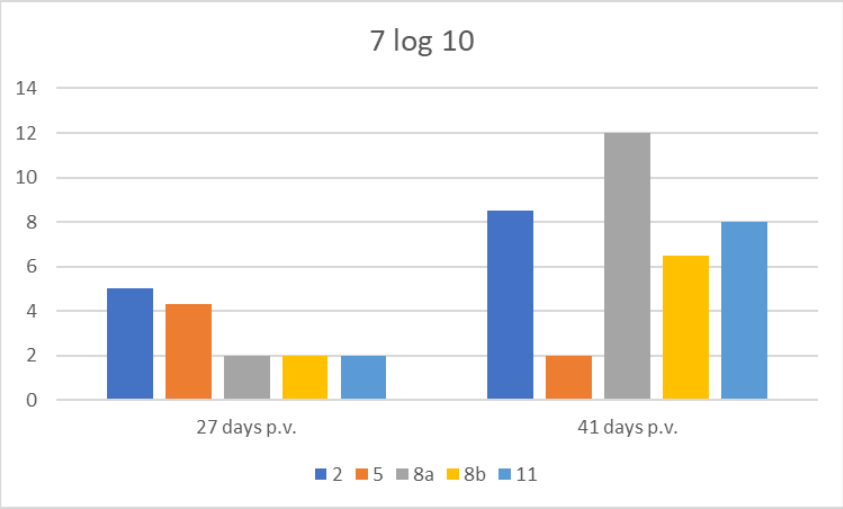
Diagnosis of Fowl Adenovirus Infections



LOHMANN
BREEDERS

Fowl Adenovirus serology





1. AGP

   Agar Gel Precipitation Test:

   group specific

2. ELISA

   Enzyme Linked Immunosorbent Assay:

   group specific

3. IFT

   Immunofluores-cent Test:

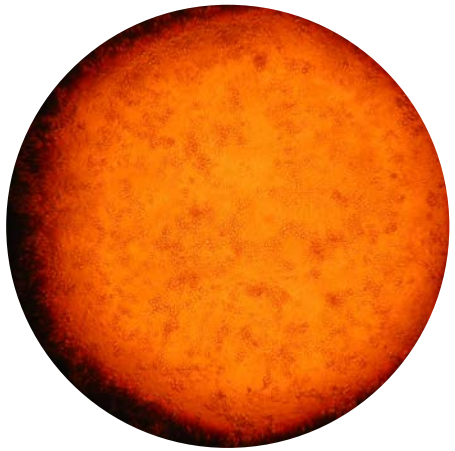
   group specific

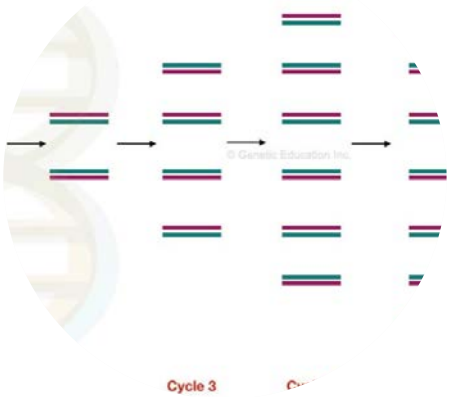
4. VN

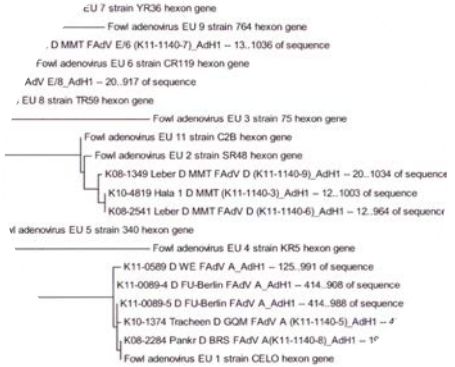
   Virus Neutralization:

   serotype specific

Fowl Adenovirus Detection





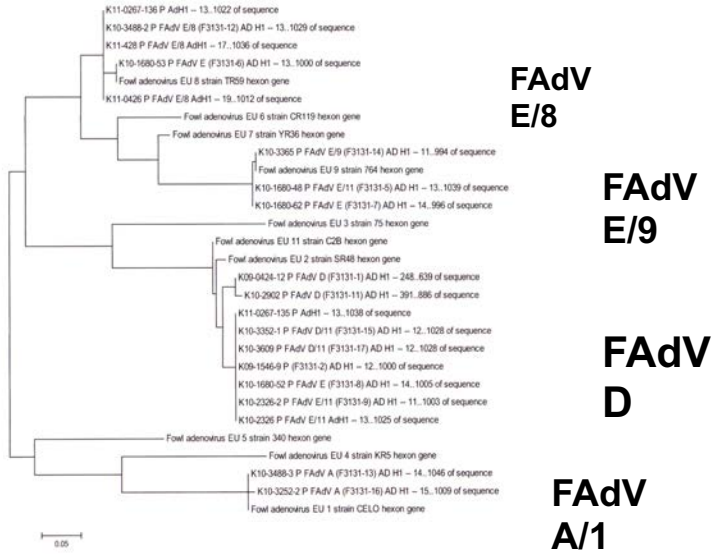


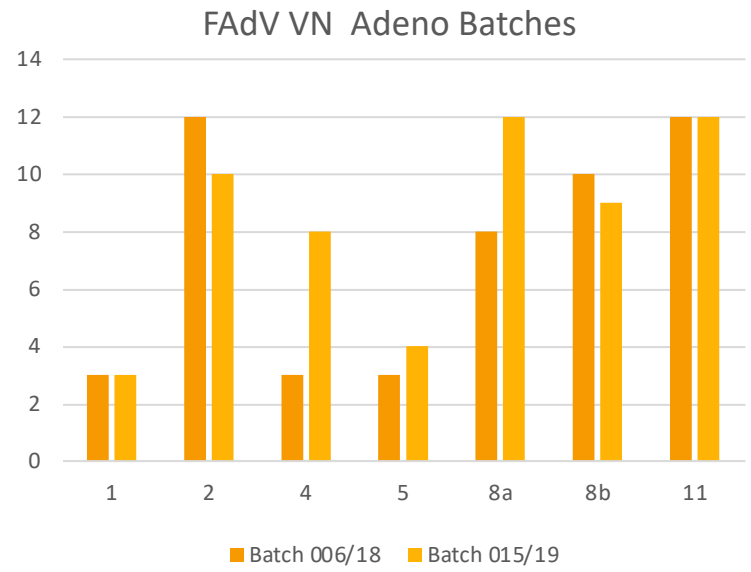
- Virus isolation in chick-embryo liver cells
- Strain identification by PCR
- DNA sequencing
- high-resolution melting (HRM-) curve analysis

LOHMANN
BREEDERS

Prevention of Fowl Adeno Infections

- **Typing of FAdV relevant field strains essential for production of autogenous vaccines.**
- **Phylogenetic trees can help to understand the epidemiological relatedness of various field isolates**

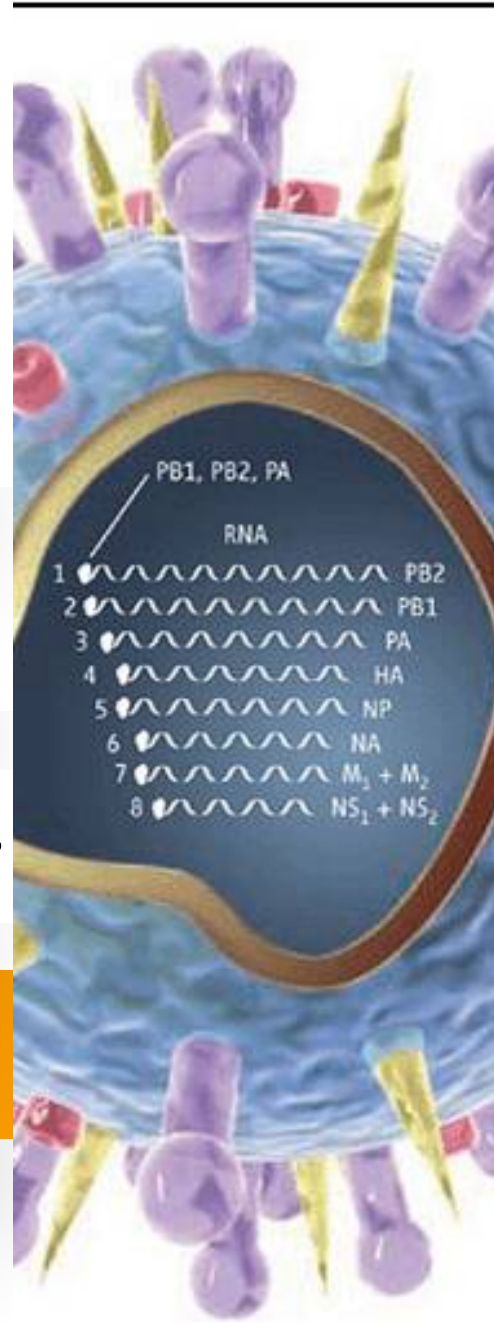




LOHMANN
BREEDERS

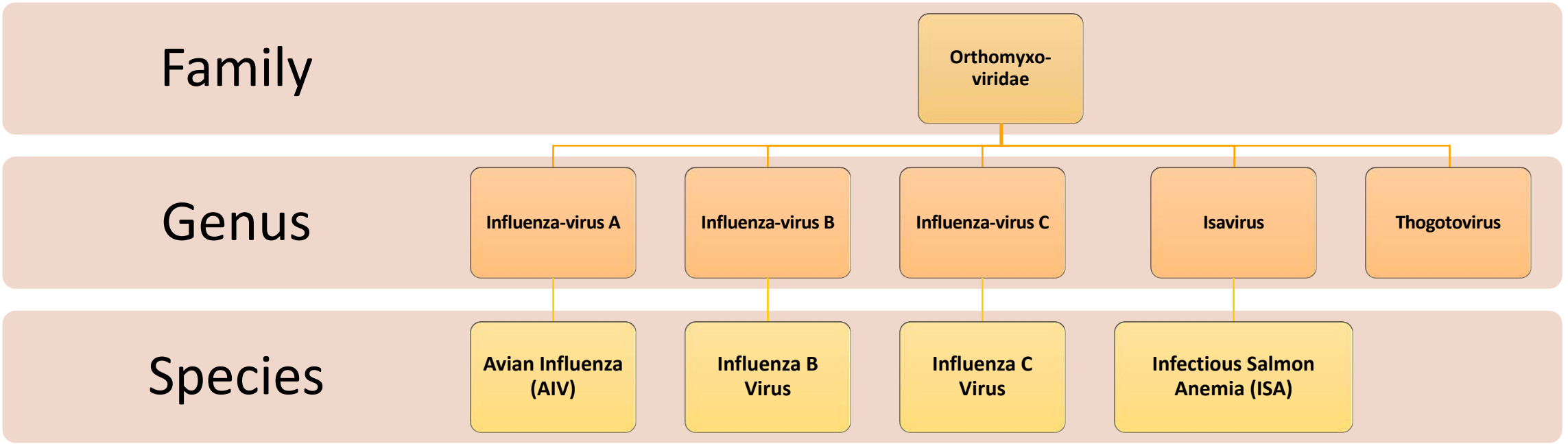
Pathogen identification and vaccine vs field strain: some examples for diagnostic challenges

- Mycoplasma
- Tumor diseases
- Infectious Bronchitis
- **Avian Influenza**



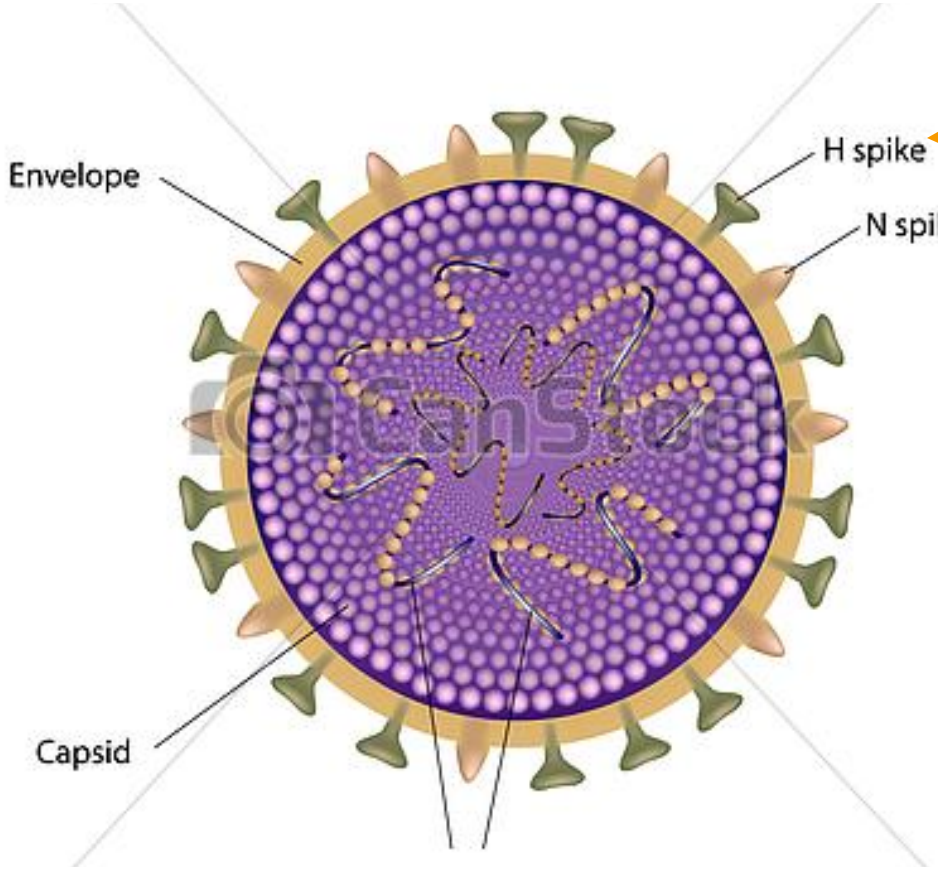
eprinted with permission from Sc

Avian Influenza: Virus Classification



LOHMANN
BREEDERS

Avian Influenza Viruses



The are currently 16 different Hemagglutinin subtypes known

H1 – H16 (17+18)

The are currently 9 different Neuraminidase subtypes known

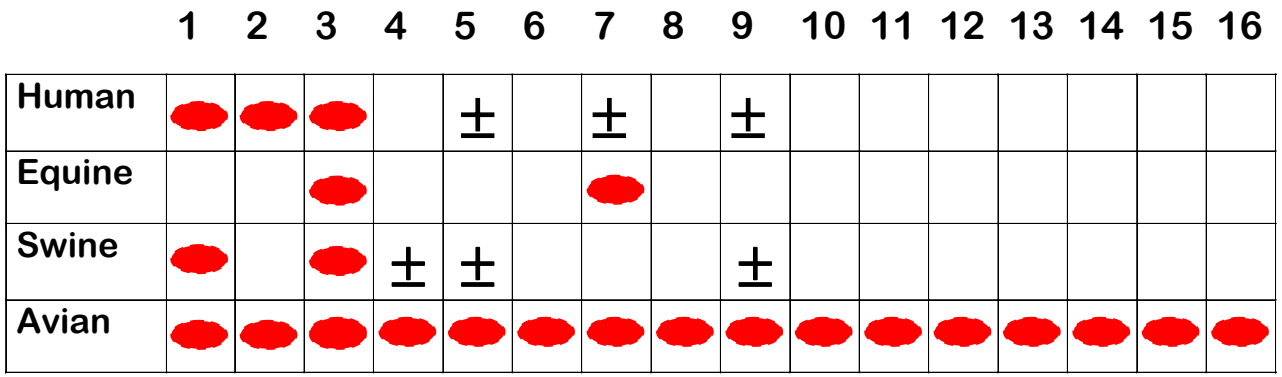
N1 – N9

Potentially there is any combination from H and N

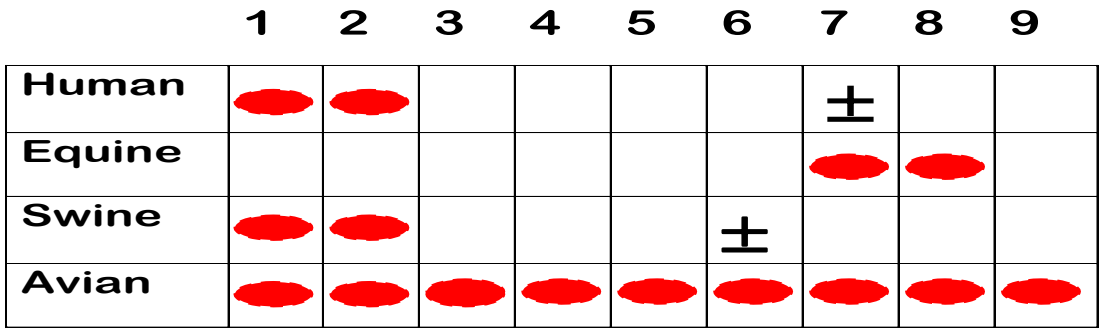
= 144

LOHMANN BREEDERS

Species prevalence of Influenza Type A viruses



Hemagglutinin (H) subtypes

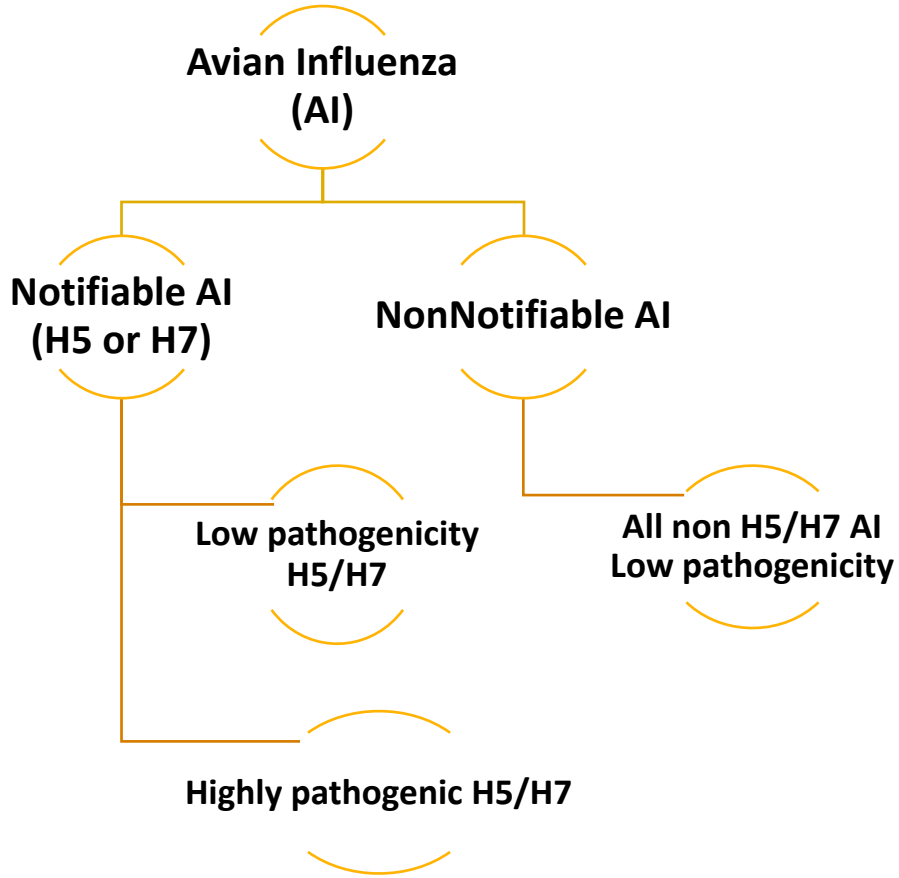


Neuraminidase (N) subtypes

H17N10 and H18N11 only been found in bats

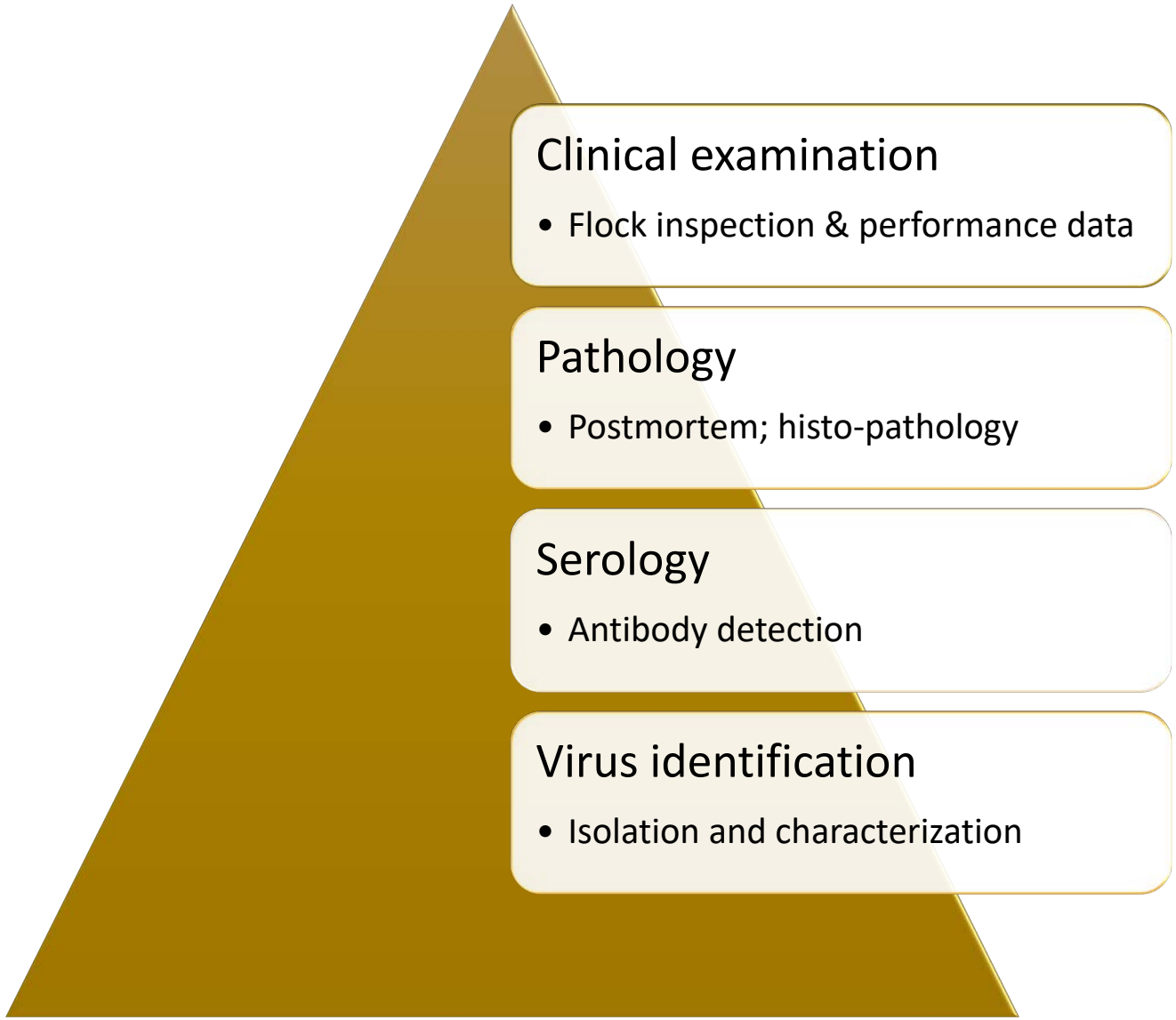
What is Avian Influenza?

Avian influenza, known informally as **avian flu** or **bird flu**, is a variety of infections caused by Influenza A viruses adapted to birds. It is initially known as **fowl plague** and was first described in 1878 in Italy



LOHMANN
BREEDERS

Diagnosis of Avian Influenza

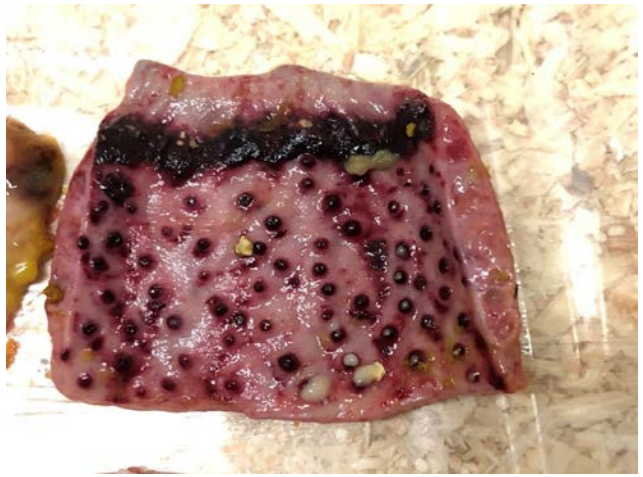


PLAI vs HPAI: Clinical signs

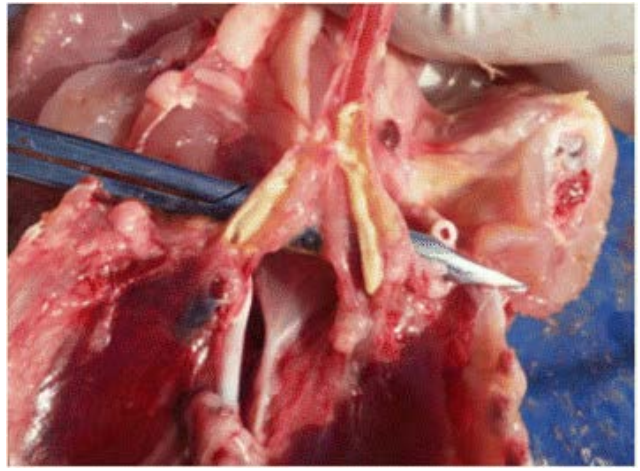
LPAI	HPAI
Lethargy	Apathy
Mild Respiratory signs; sinusitis	Stop in water and feed intake
Drop in egg production	Flock is ‚silent‘
Discoloration of eggshells, rough and misshapen eggs	High mortality (upto 100%)
Usually, moderate mortality	

LPAI: Pathology

Hemorrhages in proventriculus



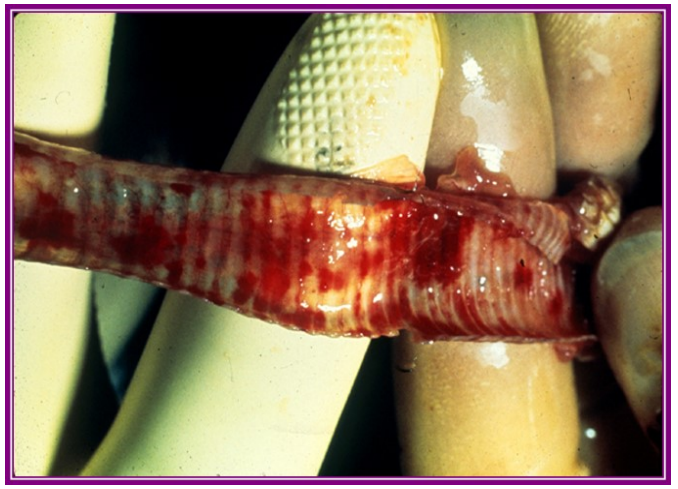
Tracheitis with fibrinous material



Blue combs

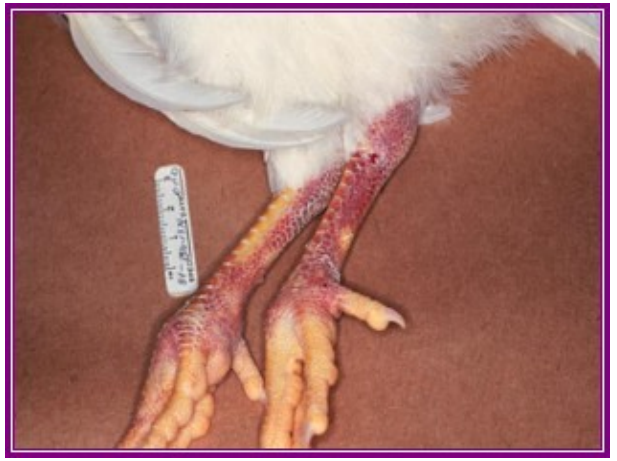
HPAI: Pathology

Infraorbital swelling; edema and/or necrosis



Severe tracheitis

Hemorrhages in proventriculus

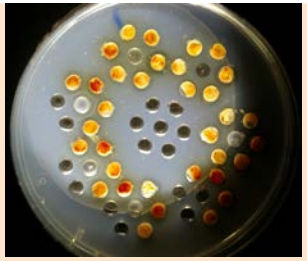


Subcutaneous hemorrhages in shank's

Serological Tests

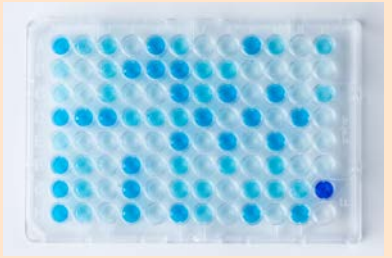
Agar gel immunodiffusion (AGID)

Very specific,
Ig M detection
Not strain specific



Enzym-linked immunosorbent assay (ELISA)

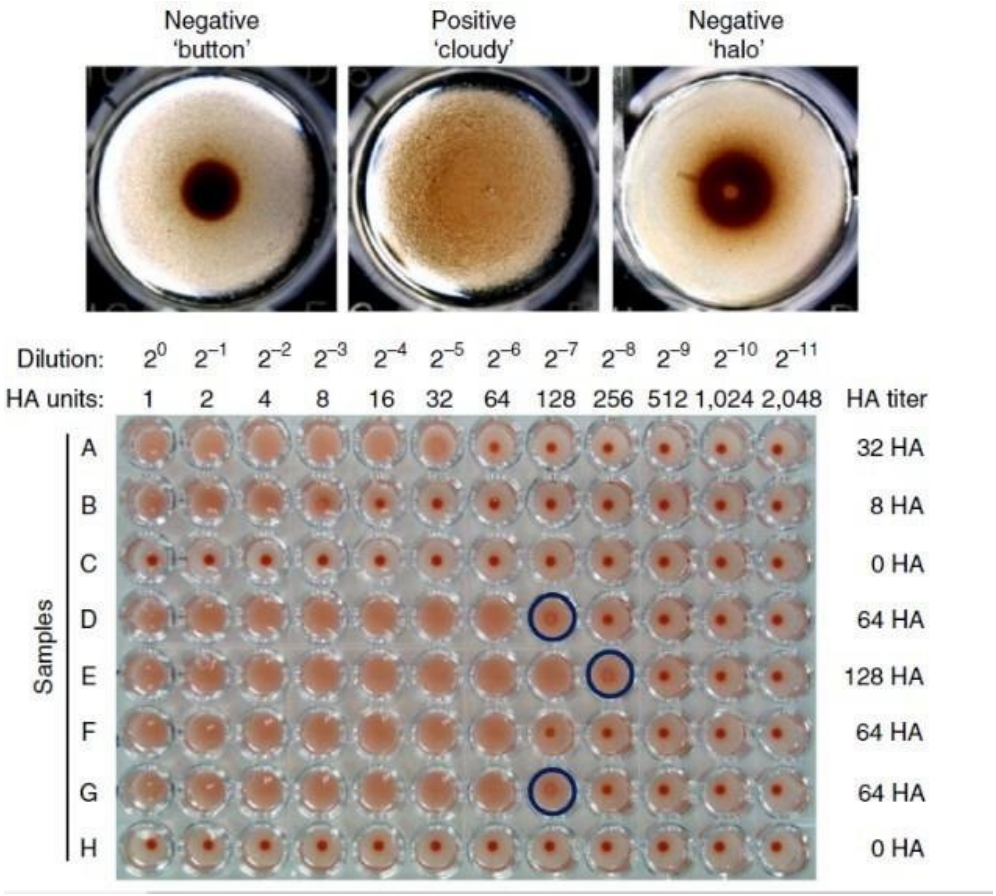
Very sensitive
less specific than AGID
Ig G detection
Not strain specific



Hemagglutination-inhibition test (HI) & neuraminidase-inhibition (NI)

High sensitivity
Not practical to use as a screening test
used to identify specific H and N antibodies in serum

Avian Influenza: Serology



Note!

- Serology for Avian Influenza could only be used to monitor infections with Low Pathogenic Avian Influenza (LPAI)
- Suspicion of Highly Pathogenic Avian Influenza has to be investigated immediately by PCR

Virus detection and identification

Virus isolation

SPF embryo culture by inoculating 9-10 days old embryos into the allantoic cavity; observe mortality; Check for hemagglutinating activity (HA); takes several days/weeks; multiplication of infectious virus!

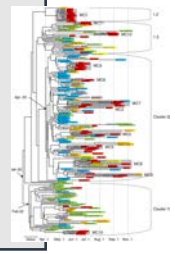
Antigen detection

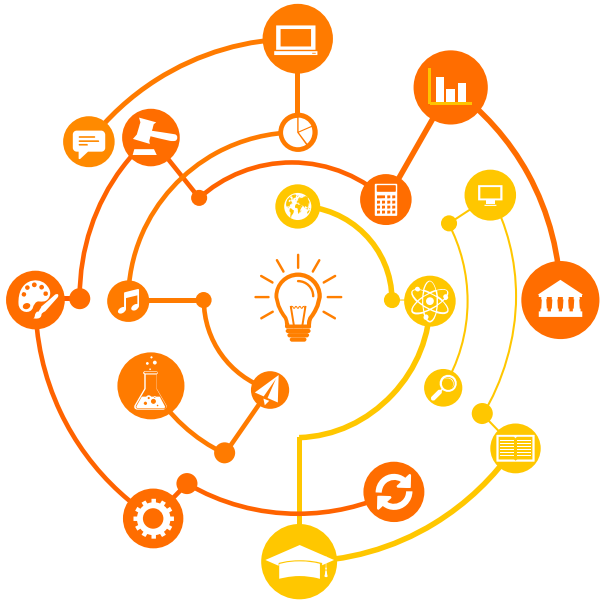
- Antigen Capture Tests
- RT-PCR

Most rapid and sensitive methods for detection of infections!

Molecular typing

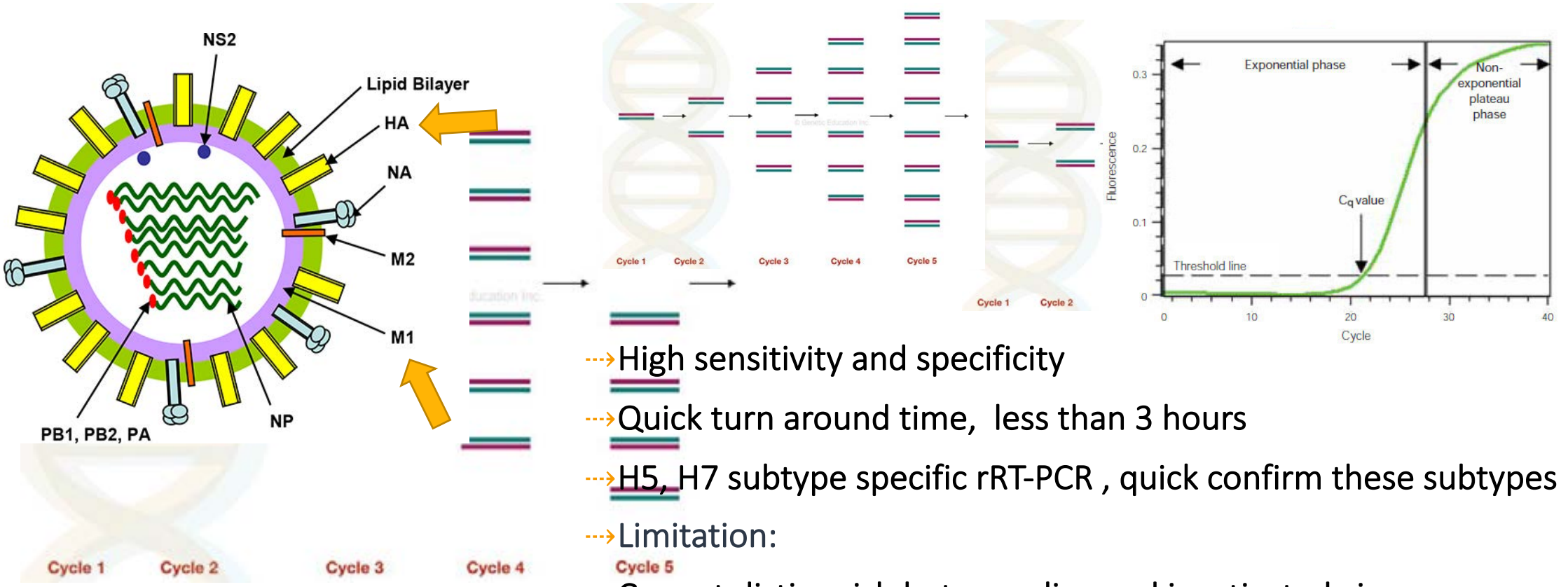
Sequencing methods to identify lineages and phylogenetic trees





LOHMANN
BREEDERS

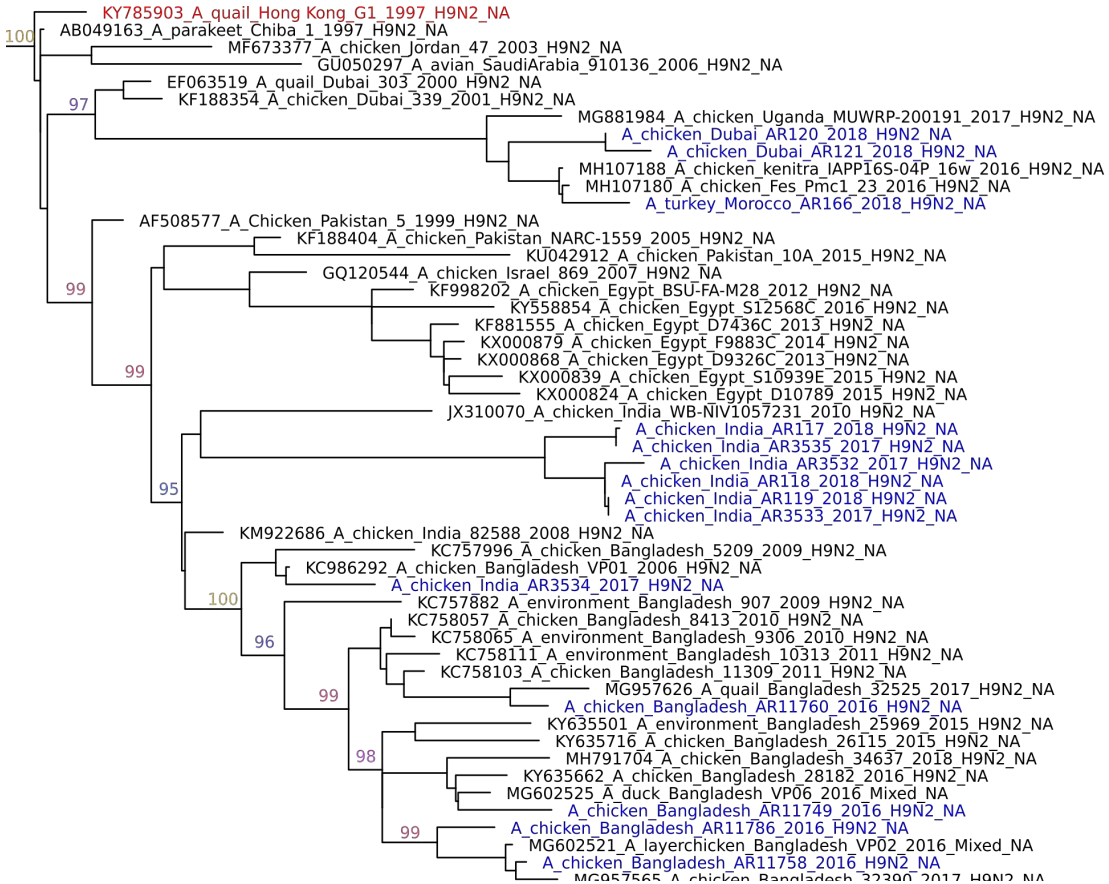
RT-PCR: Avian Influenza



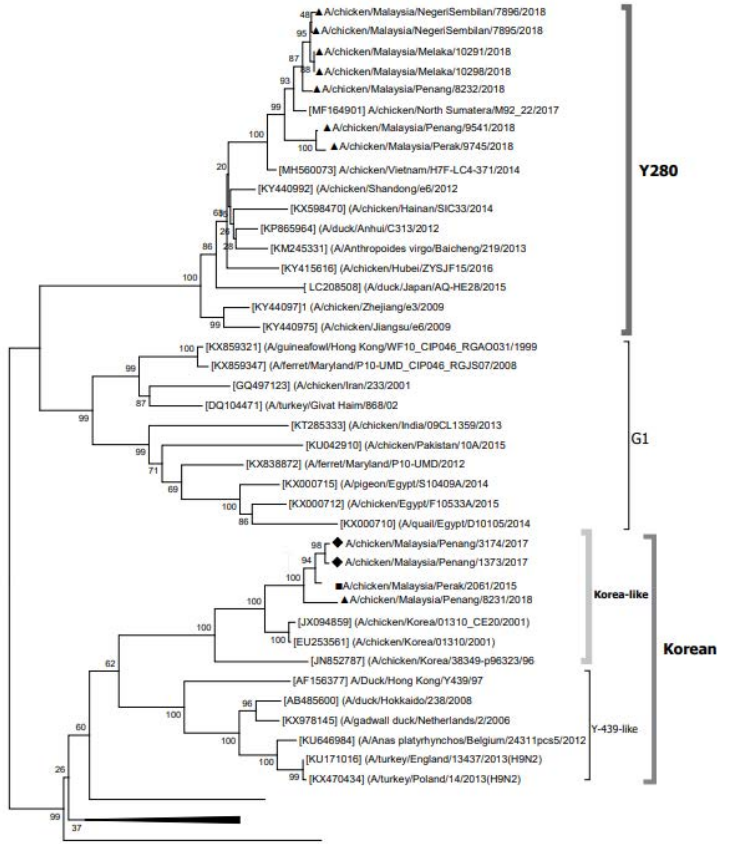
- High sensitivity and specificity
- Quick turn around time, less than 3 hours
- H5, H7 subtype specific rRT-PCR , quick confirm these subtypes
- Limitation:
- Cannot distinguish between live and inactivated virus

LOHMANN BREEDERS

Avian Influenza: Phylogenetic analysis



G1 lineage



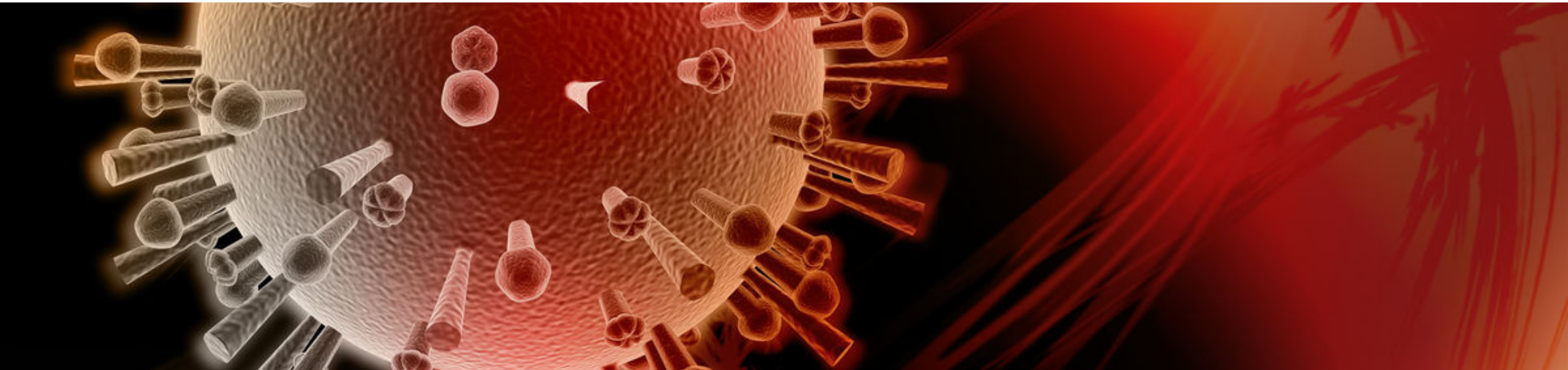
LOHMANN BREEDERS

Differentiate Infected from Vaccinated Animals (DIVA)

- The use of a DIVA principle could be a tool to prevent poultry populations at risk against Avian Influenza
- Inactivated vaccines have been devleoped in the past using different Neuraminidase subtypes, but a widely use of diagnostic methods is currently unrealistic.
- New generations of vaccines against H5 have been developed that allow DIVA monitoring as they only induce antibodies against the H5 antigen.
- Screening for antibodies against the conserved matrix or nucleoprotein allows detection of any field infection using routine commercially available ELISA tests.
- RT-PCR using primers for the conserved Matrix gene will not be able to detect a DIVA vaccine that only expresses the H5 hemagglutinin gene.

LOHMANN
BREEDERS

Future Challenges



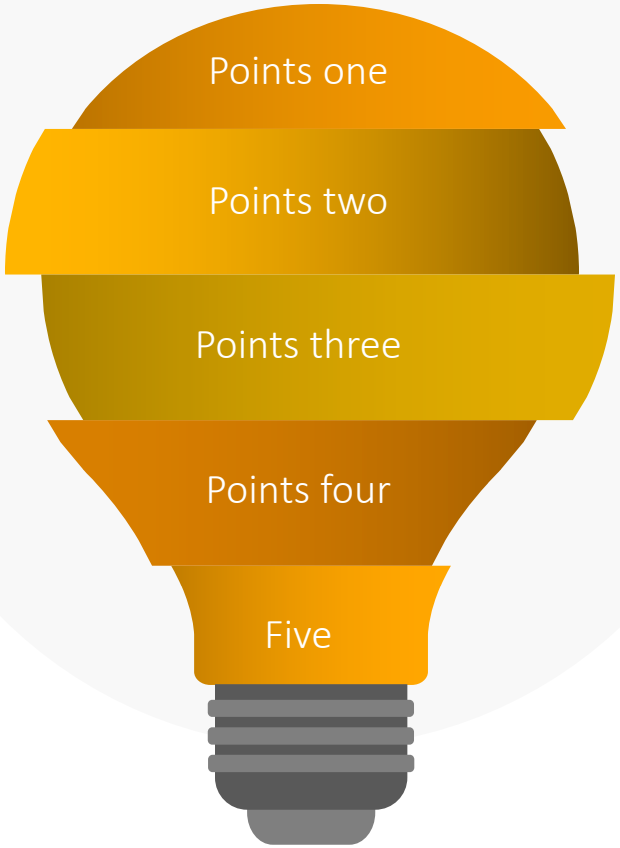
Future challenges in Poultry Diagnostics?

Sampling

Advanced, more sensitive and rapid sampling tools

Lab submission

Safe, approved method; enable import permission



On-Site-Testing

New / improved diagnostics for onsite testing

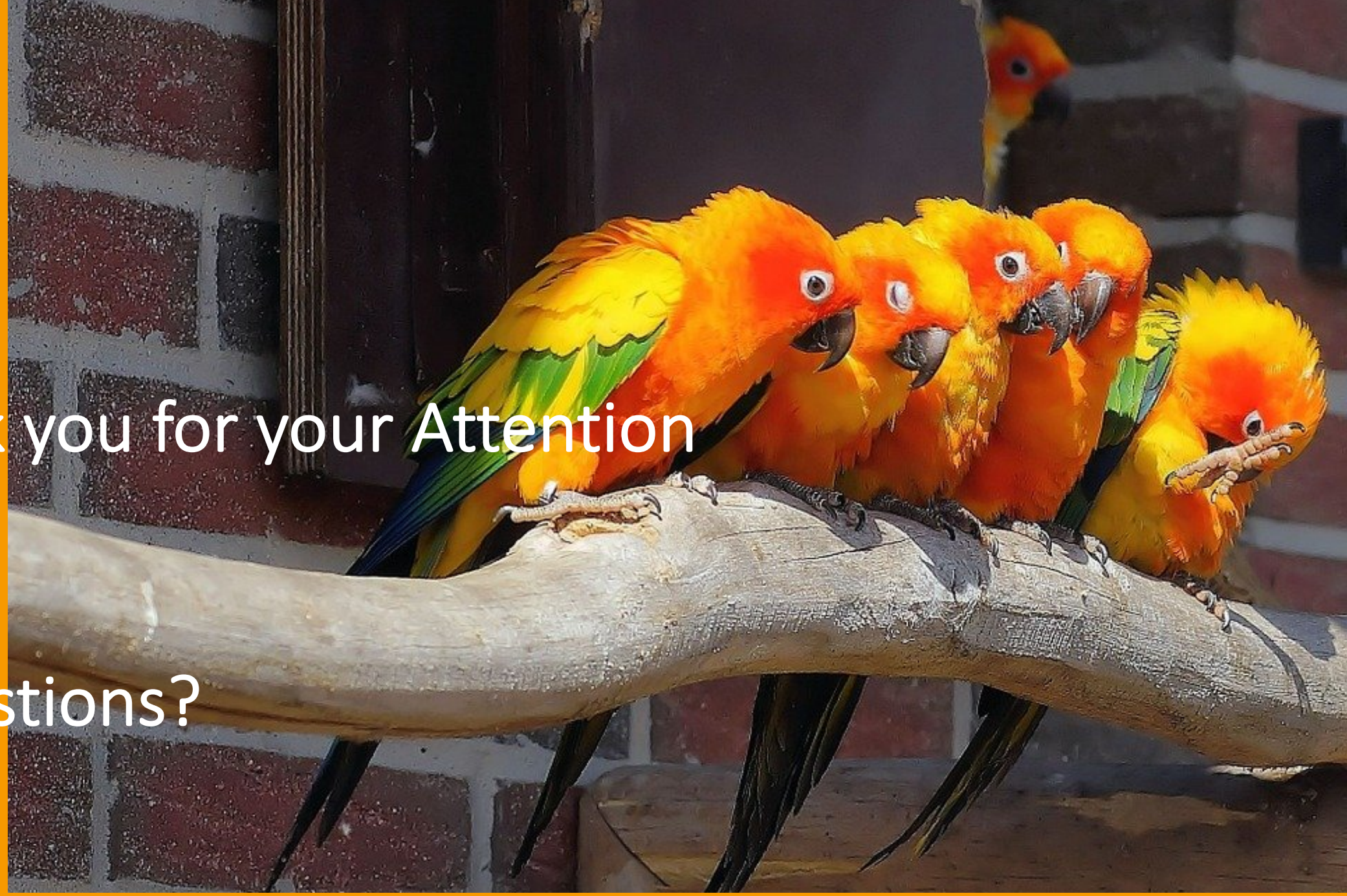
Pathogen identification

Rapid, affordable method for pathogen sub-typing

Vaccines

Vaccine technologies allowing realistic DIVA principle

LOHMANN
BREEDERS



Thank you for your Attention

Questions?