UNIVERSITY OF LIVERPOOL

Poultry respiratory-reproductive pathogens: influence of sampling techniques on laboratory outcomes

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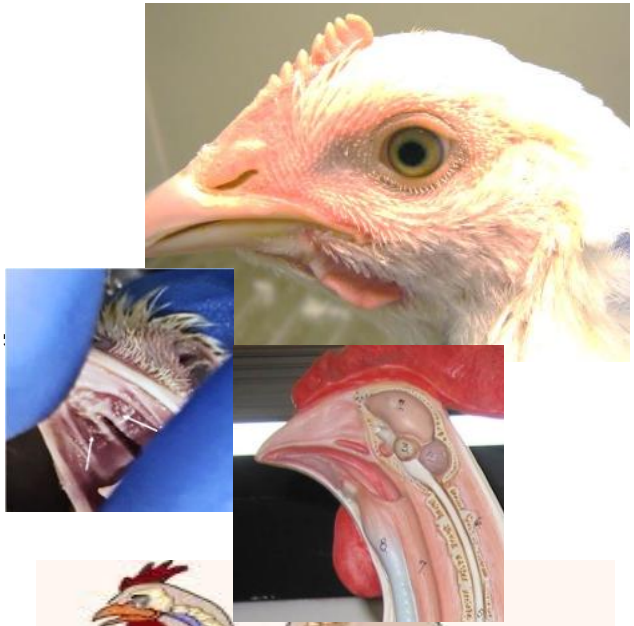
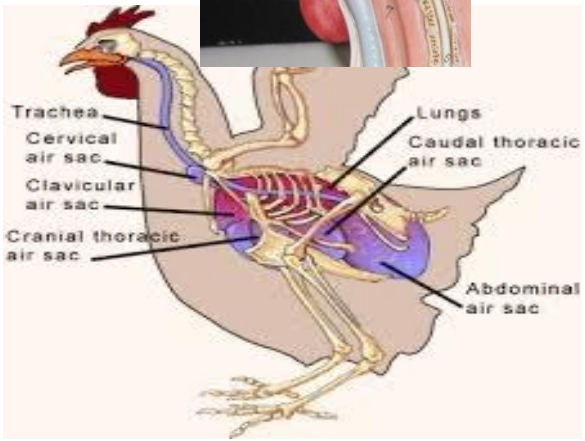
Respiratory system

- Upper respiratory tract
  - Nares
    - Mechanical filters
  - Nasal cavity
    - conchae
  - Paranasal sinus
    - Infraorbital sinuses

] micro-filteration, warms moisture

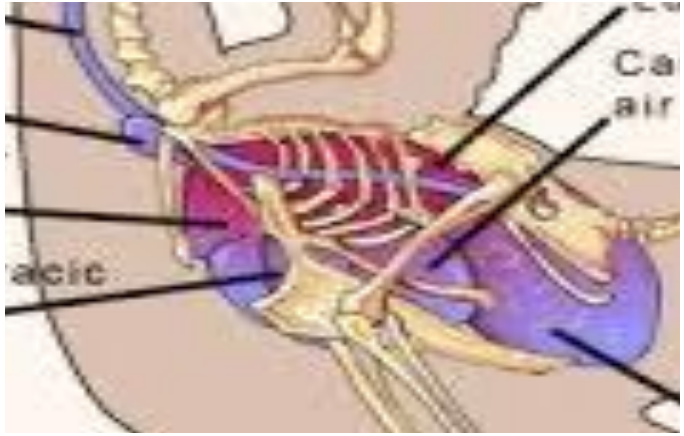


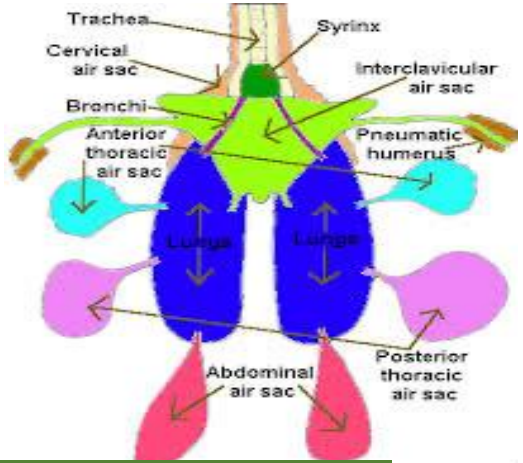
- Lower respiratory tract
  - Air passes through the *Choanae* to larynx.
  - Larynx – not connected to sound creation.
  - Larynx to trachea (complete cartilaginous rings)
  - End of trachea, bifurcates onto bronchus



Respiratory system

- Bronchi, air sacs and lungs
  - No diaphragm in birds
  - Lungs are fixed, do not expand
  - Air sacs – plays essential role
    - Number of air sacs variable depending on species
    - See diagram for the locations of air sacs
    - Thoracic and abdominal muscle movements allows inspiration and expiration of air and movements





Important & common chicken respiratory-reproductive pathogens

Pathogens	Respiratory	Reproductive
Infectious bronchitis virus (IBV)	+++	+++
Avian metapneumovirus (aMPV)	++	+
Infectious laryngotracheitis virus (ILTV)	+++	+
Mycoplasma gallisepticum (Mg)	+++	++
Mycoplasma synoviea (Ms)	++	+++
Others …		

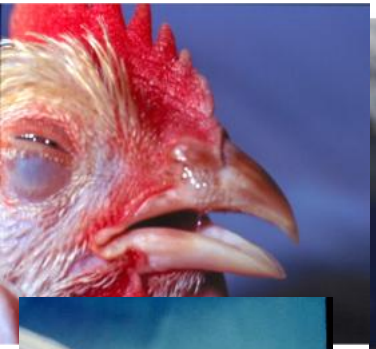
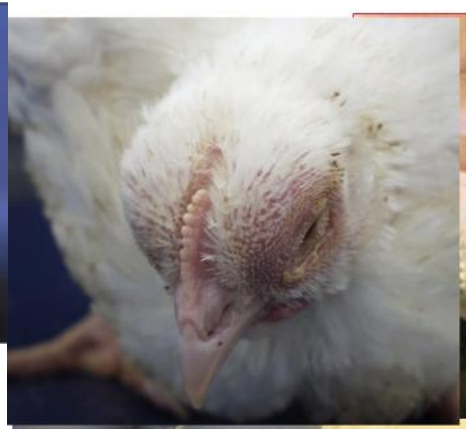
Chicken respiratory-reproductive viruses: missing diagnosis due to similar clinical signs and lesions

- Nasal and ocular discharges
- Wheezing, rales
- Sneezing, Coughing
- Gasping
- Head-shaking/scratching
- Mortality and morbidity, variable
- Abdominal breathing
- Wheezing
- Lameness

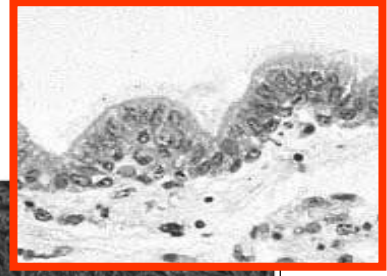
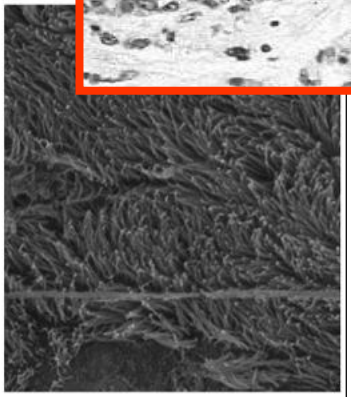
- Conjunctivitis
- Sinusitis
- Rhinitis
- Laryngitis
- Tracheitis
- Tracheal plug
- Pneumoniae
- Lung congestion
- Air-sacculitis
  - Mild
  - Moderate
  - Severe
- Fibrinous air-sacculitis

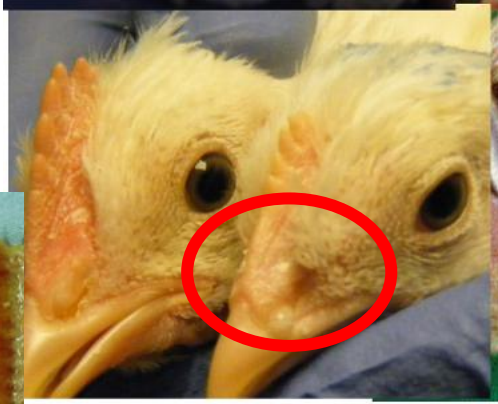
- Mortality
- Reduced egg production
- Reduced egg quality (external and internal)
- Reduced number eggs set
- Reduced hatchability
- No Vertical transmission …

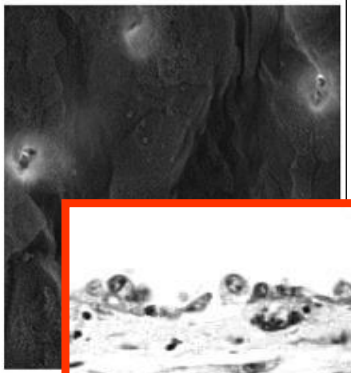
Chicken respiratory viruses: missing diagnosis due to similar clinical signs and lesions

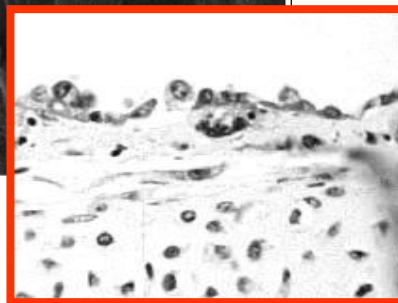









M-Movie0003.avi

Respiratory – shifting ‘complex’ to ‘supercomplex’

Simple

to

Complex
- *E coli*
- Mg
- NDV
- etc

respiratory disease

- Variant IBVs
- H9N2
- H5?
- ILT
- Ms
- ORT
- Immuno-suppression
  - Vit A, E
  - Mycotoxins
  - IBDV
  - CAV

Sugiyama, J. Vet. Med. Sci. (2006)

Chicken respiratory viruses: inappropriate sampling techniques and over-relying on single diagnostic approaches

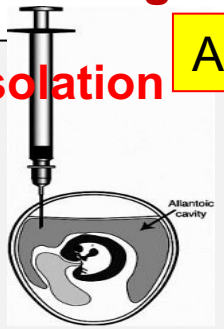
Serology

AGPT; SNT

HI

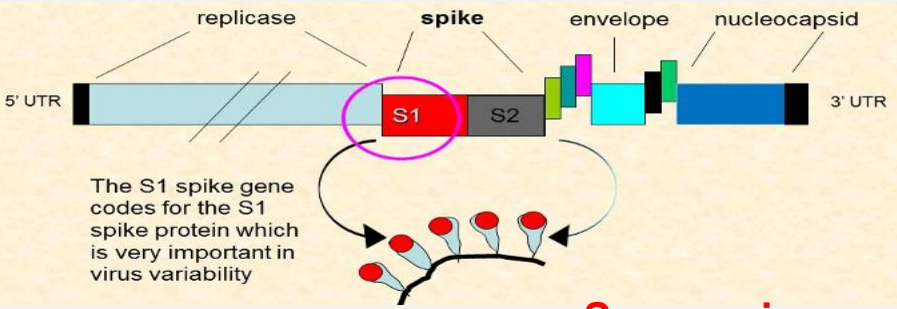
ELISA

Virus isolation

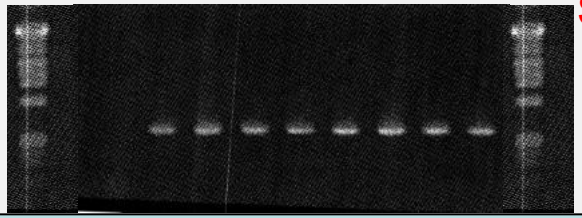


Antigen detection

Molecular methods: PCR and rt-RT-PCR



Sequencing and genotyping



3

- HPAI
- LPAI
- NDV
- IBV
- ILTV
- aMPV
- FP

1

2

- HPAI
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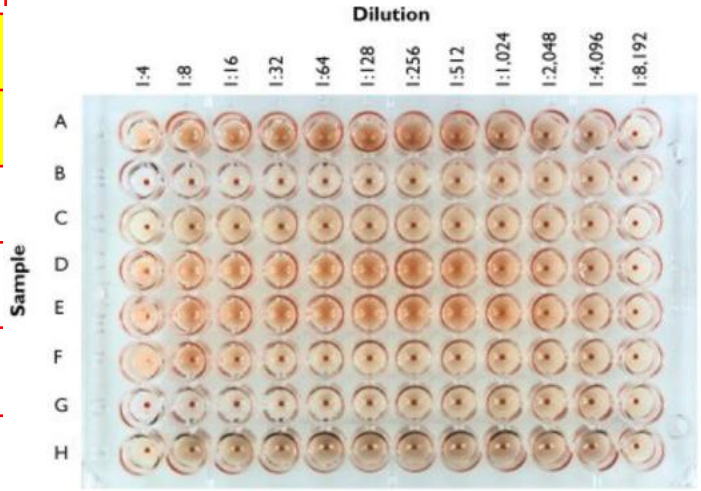
HI – Haemagglutination inhibition



HI Testing House 1

	1	2	3	4	5	6	7	8	9	10	11	12	Avg
M41					1	2	5	2					6.80
793/B						3	4	2	1				7.10

HI Testing House 2

	1	2	3	4	5	6	7	8	9	10	11	12	Avg
M41						3	5	2					6.90
793/B					1	5	3	1					6.40

HI Testing House 3

	1	2	3	4	5	6	7	8	9	10	11	12	Avg
M41						6	4						6.40
793/B					3	3	3	1					6.20

IBV-HI antibody titers in broiler flocks suffering from kidney damages.

Flocks	Mean titers (log_2)			
	Qx	M–41	D–274	4/91
1	✓ 11.00	5.80	4.70	7.00
2	✓ 11.00	7.00	4.64	7.55
3	✓ 9.70	6.50	5.20	7.20
4	✓ 10.82	6.18	5.18	7.00
5	✓ 11.00	8.09	6.91	9.00
6	✓ 11.00	6.70	5.10	6.70
7	9.78	9.78	8.78	9.89
8	✓ 11.00	7.75	5.25	7.50
9	✓ 10.50	5.33	4.50	5.17
10	✓ 10.14	4.86	4.00	5.00
11	9.50	8.33	5.83	9.67
12	8.73	7.55	5.91	8.00
13	9.80	8.70	6.70	8.80
14	10.75	9.38	6.75	9.88

Lounas et al. 2018

Mean IBV HI antibody titres in IS/885-infected broiler chicks using homologous (IS/885) and heterologous (M41, 793B) antigens.

Days post infection	Infected group			Control		
	M41	793B	IS/885	M41	793B	IS/885
0	6.2±0.6[a]	5.8±0.5[a]	3.5±0.2[b]	6.2±0.6[a]	5.8±0.5[a]	3.5±0.2[b]
3	5.1±0.7[a]	5.0±0.2[a]	2.6±0.1[a]	5.1±1.1[a]	5.5±0.8[a]	3.1±0.1[b]
6	2.4±0.6	1.3±0.3	2.1±0.2	3.0±0.3 [a]	3.3±0.5[b]	1.7±0.3[a]
9	1.6±0.8	1.4±0.2	1.8±0.2	1.6±0.8	2.3±0.6	1.1±0.2
15	3.5±0.1[a]*	3.3±0.1[a]*	4.0±0.2[a]*	1.3±0.4	0.7±0.1	1.0±0.0
28	3.7±0.1[a]*	3.5±0.1[a]*	4.5±0.1[c]*	0.7±0.2	0.4±0.1	0.1±0.1

Experimental infection of IS/885/00-like infectious bronchitis virus in specific pathogen free and commercial broiler chicks

Enez Awad [c, b], Rajesh Chhabra [a], Anne Forrester [c], Julian Chantrey [a], Matthew Baylis [a, c], Stephane Lemiere [d], Hussein Aly Hussein [e], Kannan Ganapathy [c]

Avian (Poultry-chicken) respiratory viruses: inappropriate sampling techniques and over-relying on single diagnostic approaches

Serology

AGPT; SNT

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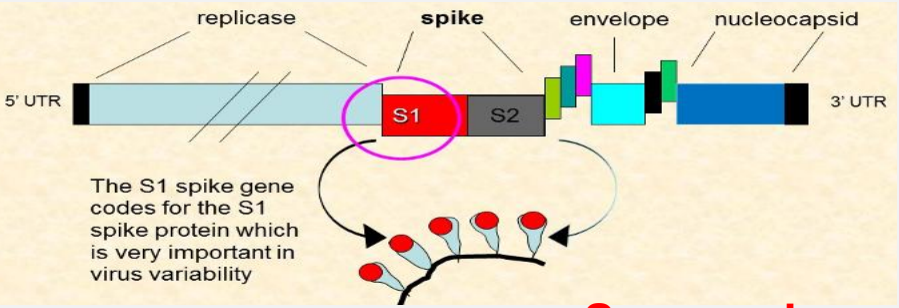
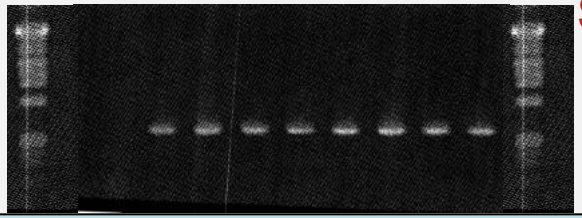


Sequencing and genotypin



3

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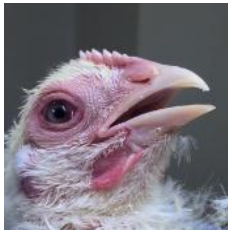
Chicken respiratory pathogens: swabs collection from birds for detection of antigens

Sampling

Live birds

https://www.hyline.com/filesimages/TechLibrary/Misc%20Docs/TU%20SER%20ENG.pdf

Tracheal



Oral (O)

Choanal (C)

Cloaca

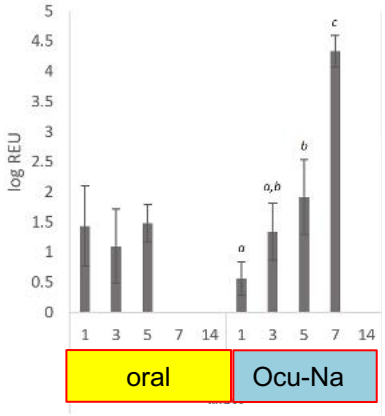
https://www.poultryimprovement.org/documents/WIAV0020.pdf

Oropharyngeal (OP)

Orophargyngeal-choanal (OPC)

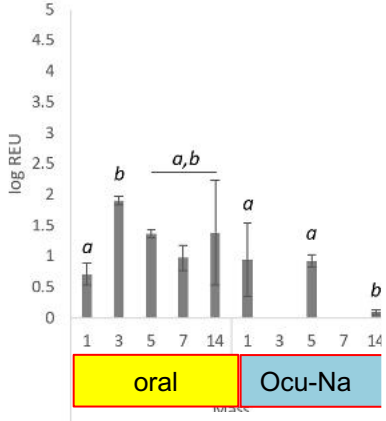
OPC & Cloacal swabs in commercial layers

OPC swabs



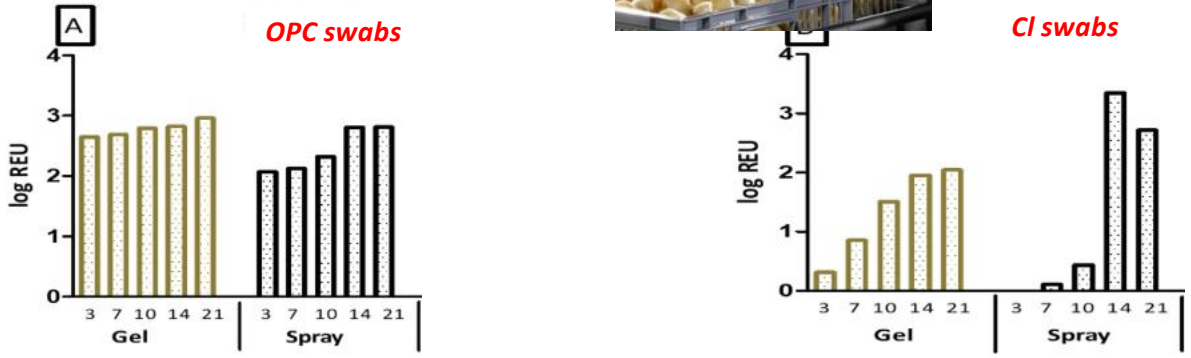
Mass

CI swabs



Mass

OPC & Cloacal swabs in commercial broilers



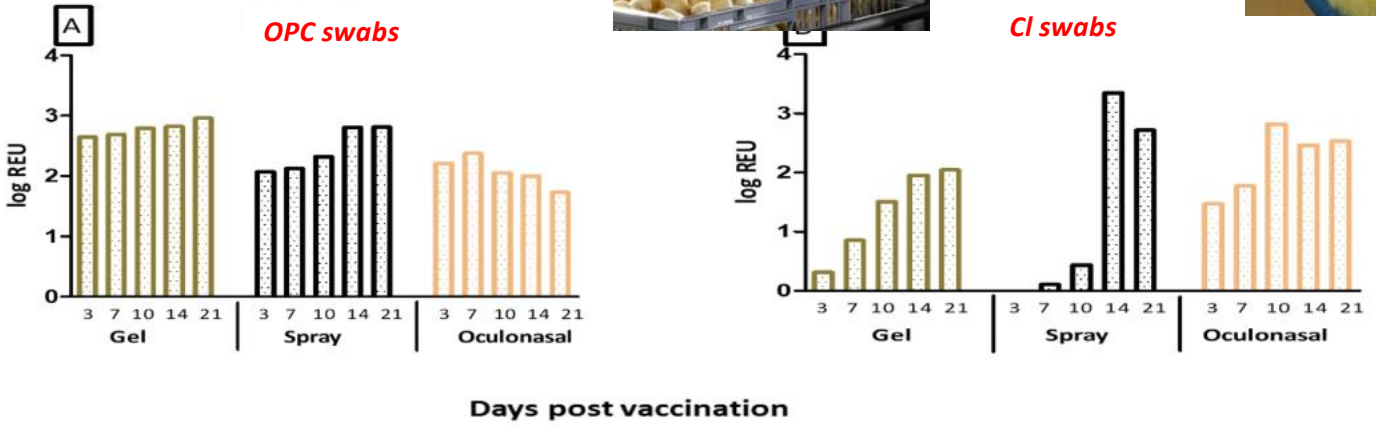
- Virus detection influenced by …
  - Strain
  - Route
- Both OPC & Cloacal swabs to monitor vaccine-take

Vaccine
Volume 41, Issue 31, 12 July 2023, Pages 4508-4524

Evaluation of protection and immunity induced by infectious bronchitis vaccines administered by oculonasal, spray or gel routes in commercial broiler chicks

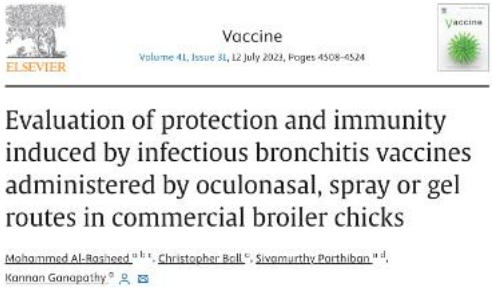
Mohammed Al-Rasheed [a, b, c], Christopher Bell [c], Sivamurthy Parthiban [a, d], Kannan Ganapathy [a]

OPC & Cloacal swabs in commercial broilers



- Virus detection influenced by …
  - Type of birds
  - Strain of virus
  - Route of inoculation

- **Both OPC & Cloacal swabs to monitor vaccine-take**



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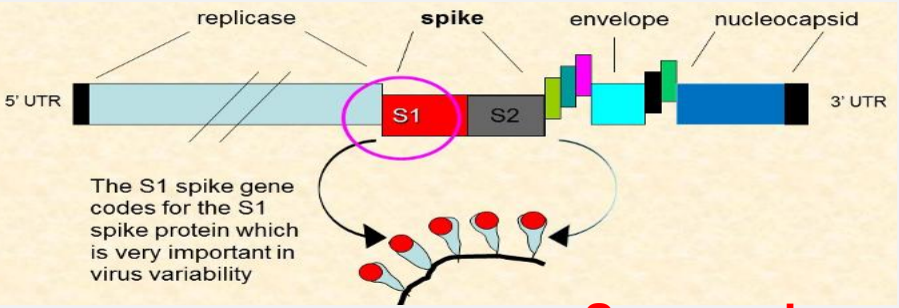

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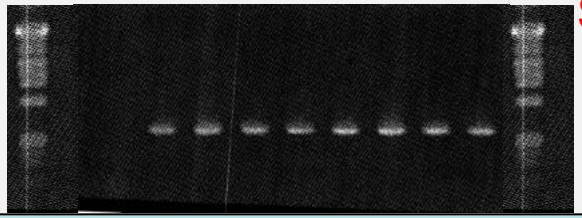

3

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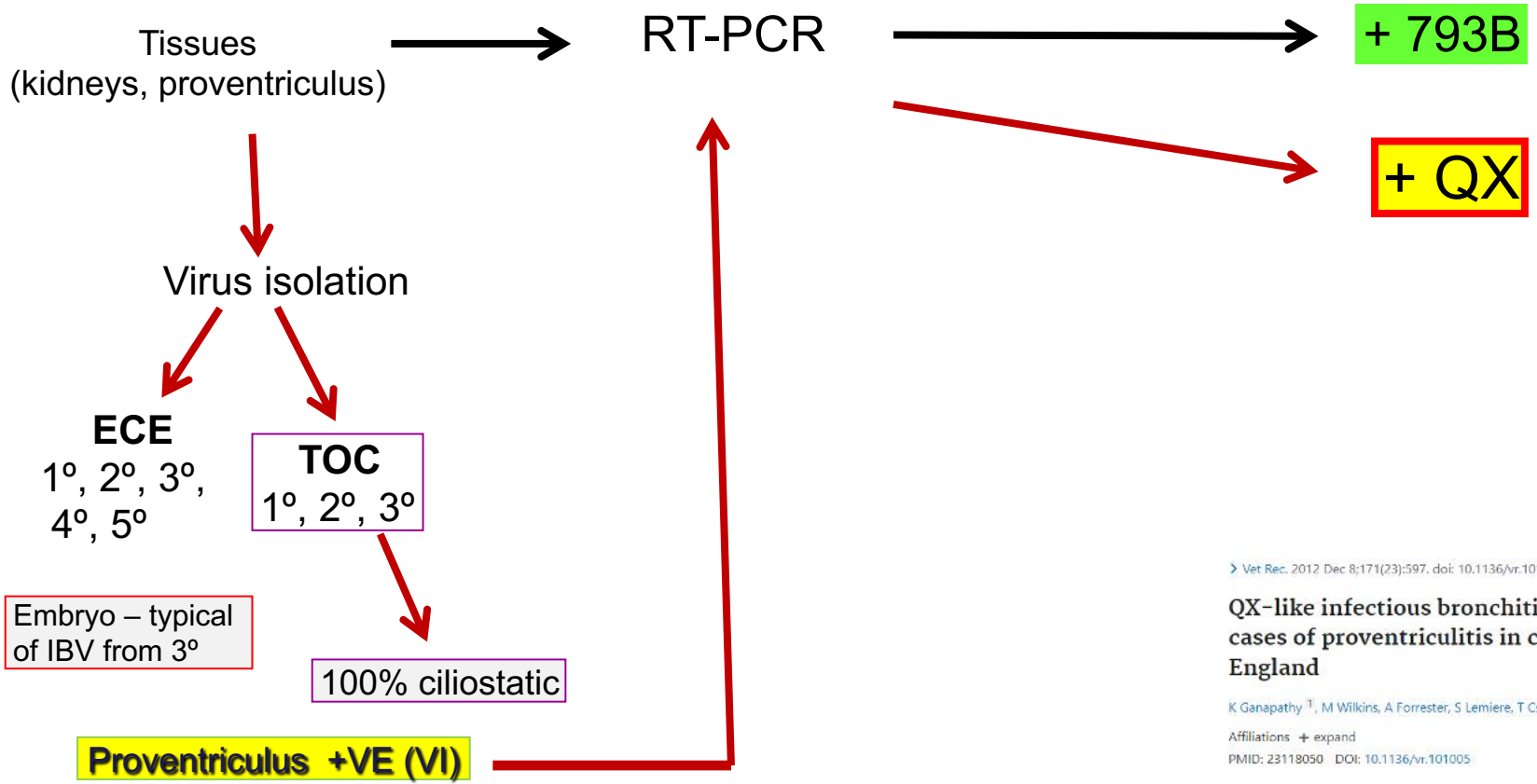
Conventional & Molecular detection & differentiation of pathogens – RT-PCR vs VI



› Vet Rec. 2012 Dec 8;171(23):597. doi: 10.1136/vr.101005. Epub 2012 Nov 1.

QX-like infectious bronchitis virus isolated from cases of proventriculitis in commercial broilers in England

K Ganapathy [1], M Wilkins, A Forrester, S Lemiere, T Cserep, P McMullin, R C Jones

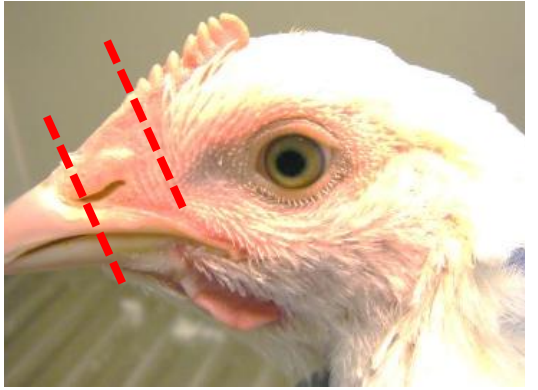
Affiliations + expand

PMID: 23118050 DOI: 10.1136/vr.101005

A comparison on the detection of aMPV by RT-PCR on samples collected on FTA cards either as tissue impression smear or inoculated with tissue supernatant

Tissue	Groups infected with aMPV subtype A or B	Methods of tissue sampling onto FTA card	Day post-infection					
			1	3	5	7	9	14
Turbinate	A	Impression[a]	-	+	+	+	-	-
		Supernatant[b]	+	+	+	-	-	-
	B	Impression[a]	+	+	+	+	+	+
		Supernatant[b]	+	+	-	+	+	+
Trachea	A	Impression[a]	-	-	+	+	-	-
		Supernatant[b]	-	+	+	-	-	-
	B	Impression[a]	-	-	+	-	-	-
		Supernatant[b]	+	+	+	-	-	+
Lung	A	Impression[a]	-	+	+	-	-	-
		Supernatant[b]	-	-	+	-	-	-
	B	Impression[a]	-	-	+	+	+	-
		Supernatant[b]	+	-	+	+	-	-

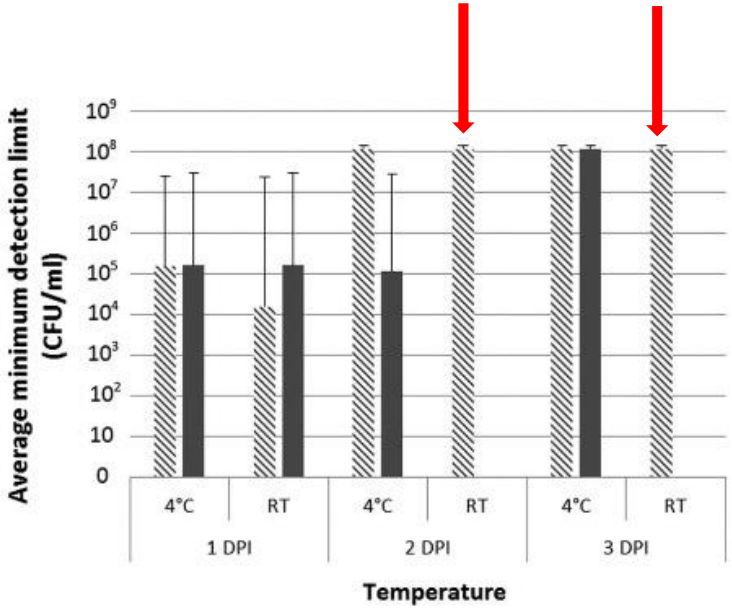
a: imprint was made by gently pressing the tissue against providing matrix area of the FAT card; b: 100 µl of the supernatants of turbinate, trachea and lung were spotted directly onto FTA card.

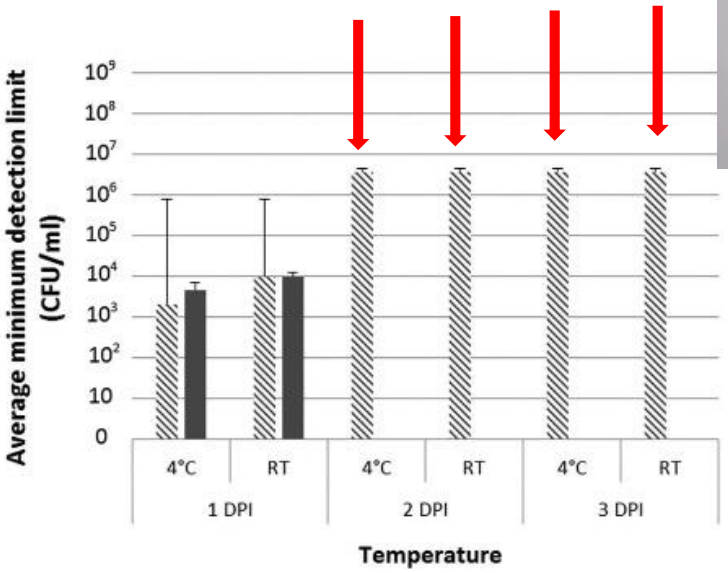


Preferred tissues & timing for detection of pathogens, eg. aMPV

ORIGINAL ARTICLES
Evaluation of Flinders Technology Associates cards for storage and molecular detection of avian metapneumoviruses
Faez Awad, Matthew Baylis, Richard C. Jones & Kannan Ganapathy
Pages 125-129 | Received 17 Jun 2013, Accepted 11 Dec 2013, Published online: 12 Feb 2014

Sampling for Mg/Ms – best to use plastic stick swabs





Plastic

Wooden

AVIAN PATHOLOGY
2020, VOL. 49, NO. 1, 106–110
https://doi.org/10.1080/03079457.2019.1675865

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Check for updates

Influences of swab types and storage temperatures on isolation and molecular detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*

Christopher Ball[a], Viviana Felice[b], Yichao Ding[c], Anne Forrester[a], Elena Catelli[b] and Kannan Ganapathy[a]

[a]Institute of Infection and Global Health, University of Liverpool, Neston, UK; [b]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia (BO), Italy; [c]College of Animal Science, Zhejiang University, Hangzhou, People's Republic of China

Conclusions

- Clinical judgement *vs* laboratory reports.
- Cross-check laboratory results.
- Use a <u>combination</u> of antibody and antigen detection methods.
- Ensure correct sampling methods.
- Appropriate interpretation of laboratory results.

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Sirorat Munyahongse, PhD
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Sam Hughes, PhD

Joseph Hinds, BBSRC Postdoc
Emily Herschell-Kelly, BBSRC Technician

Thanks for your kind attention
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