


**MEMBER STATE
(& THIRD COUNTRIES) REPORTS
FOR AVIAN INFLUENZA 2018**
Based on responses to the questionnaire
received

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AI-ND EURL

25th annual meeting of the European National Reference Laboratories for AI and ND

19-21 June 2019 – Padova - Italy



● Questionnaire 2018 - Response rate

- Invited Laboratories: 31 (30 Countries)
- Responses received: 29/31 (93,5%)
 - EU Laboratories 27/29 (93,1%)
 - Non-EU laboratories 2/2 (100%)

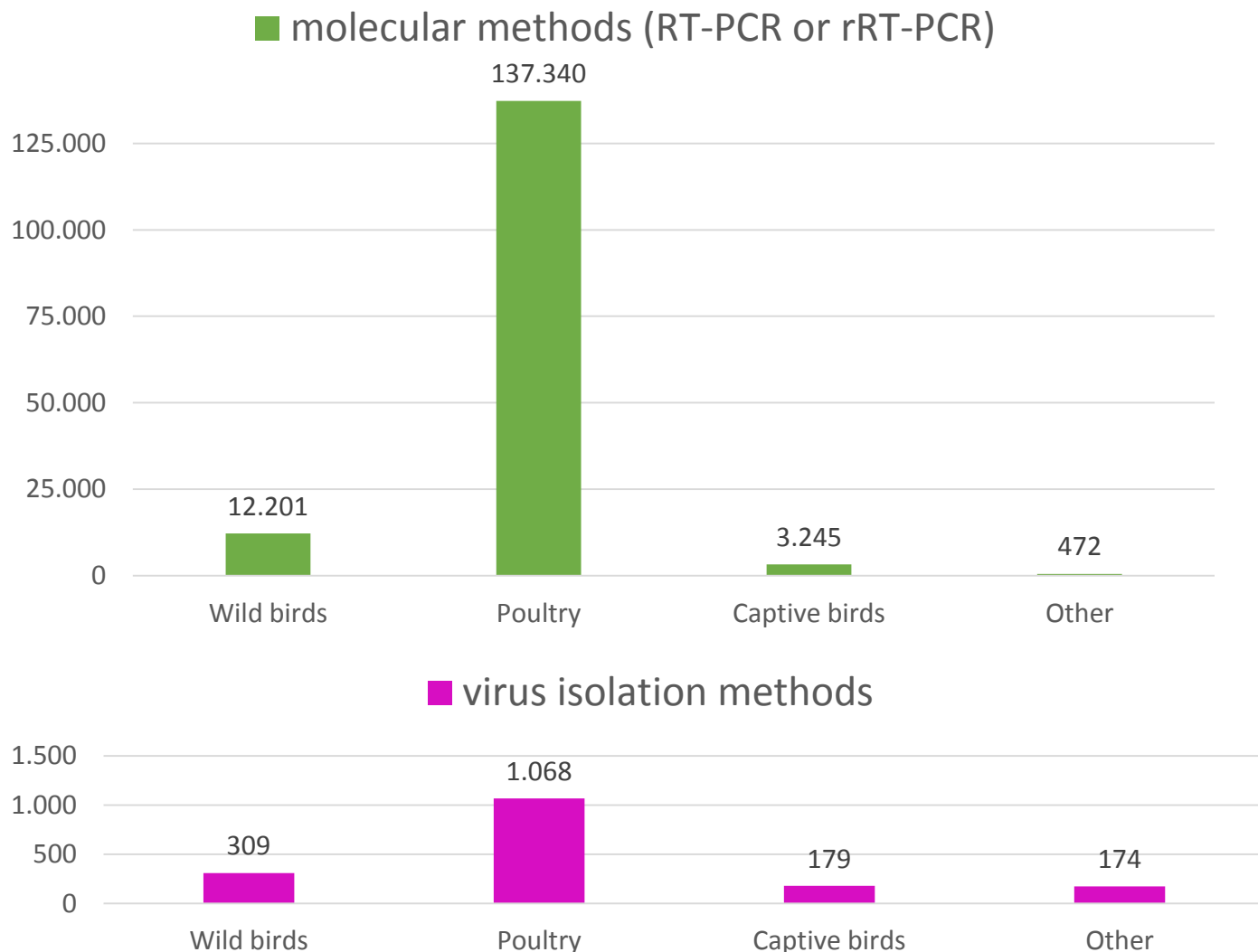
● Responding laboratories

- Austria
- Belgium
- Bulgaria
- Cyprus
- Croatia
- Czech Republic
- Denmark
- Estonia
- Finland
- France
- Germany
- Greece (Thessalonica)
- Greece (Athens)
- Hungary
- Republic of Ireland
- Italy
- Latvia
- Luxembourg
- The Netherlands
- *Norway*
- Poland
- Portugal
- Romania
- Slovakia
- Slovenia
- Spain
- Sweden
- *Switzerland*
- UK



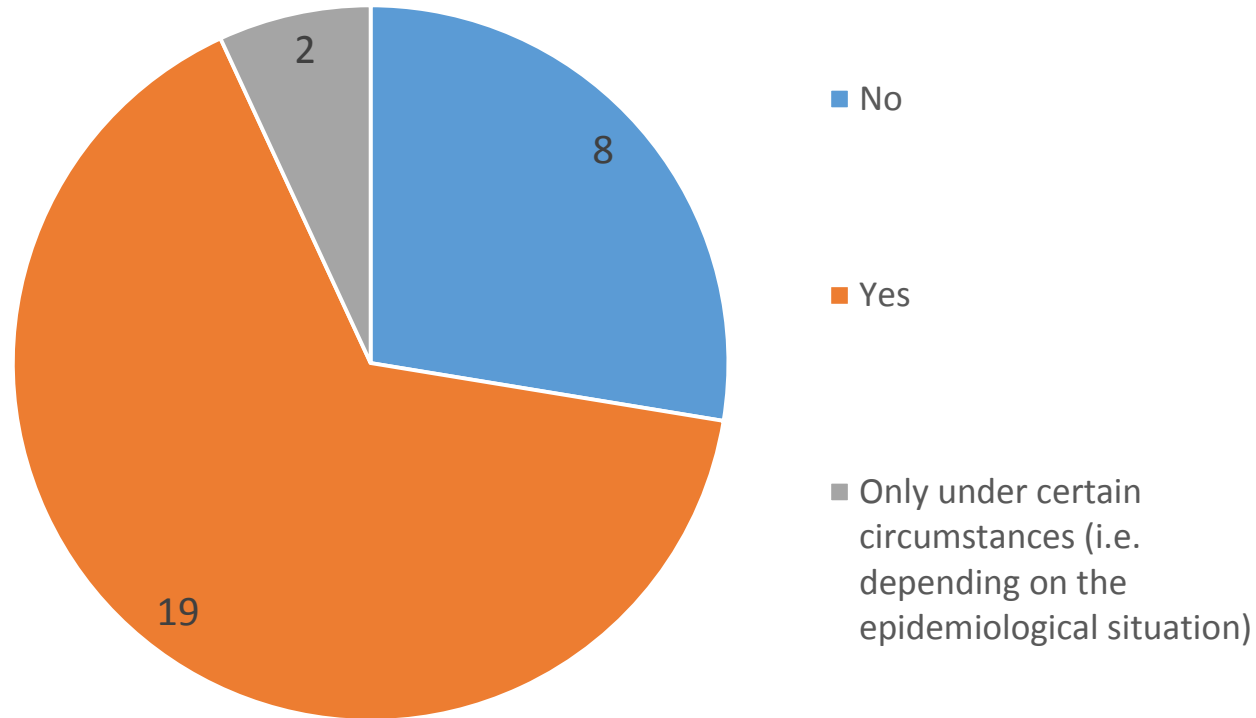
Summary of responses received

AI 1.1 How many samples from poultry or wild birds were processed by molecular methods (RT-PCR or rRT-PCR) or virus isolation methods?



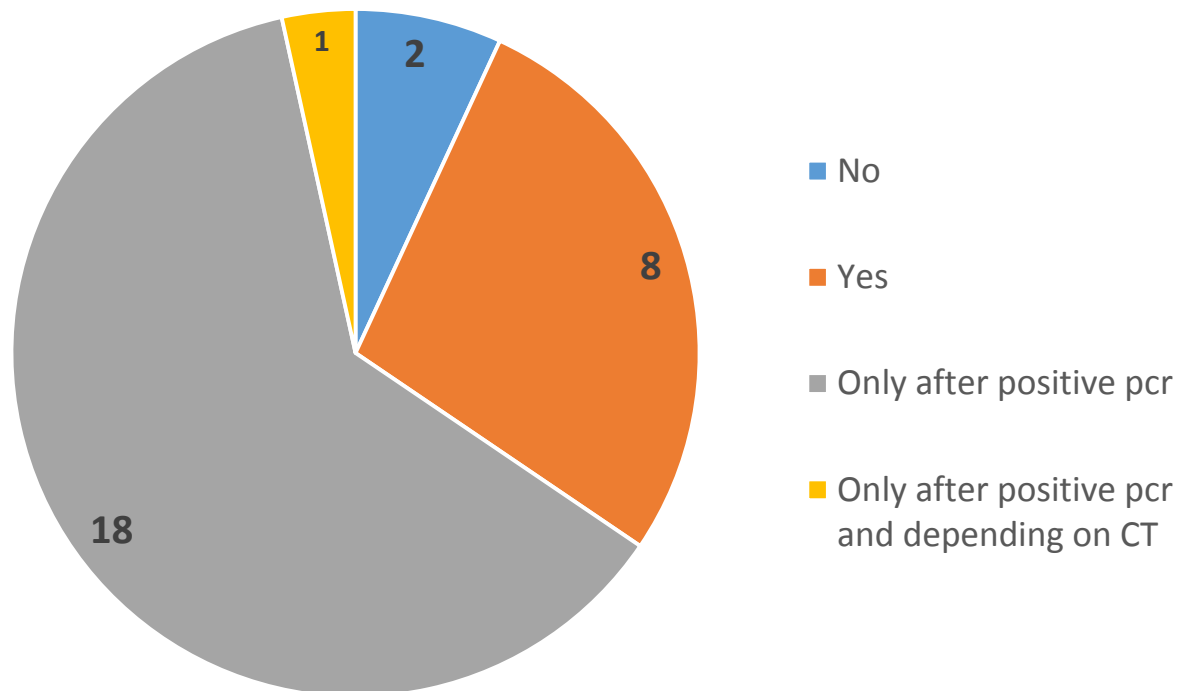
VI/RT-PCR proportion= @ 1%

AI 1.2 When a suspicion for ND is negative, is testing for AI systematically carried out for the differential diagnosis?



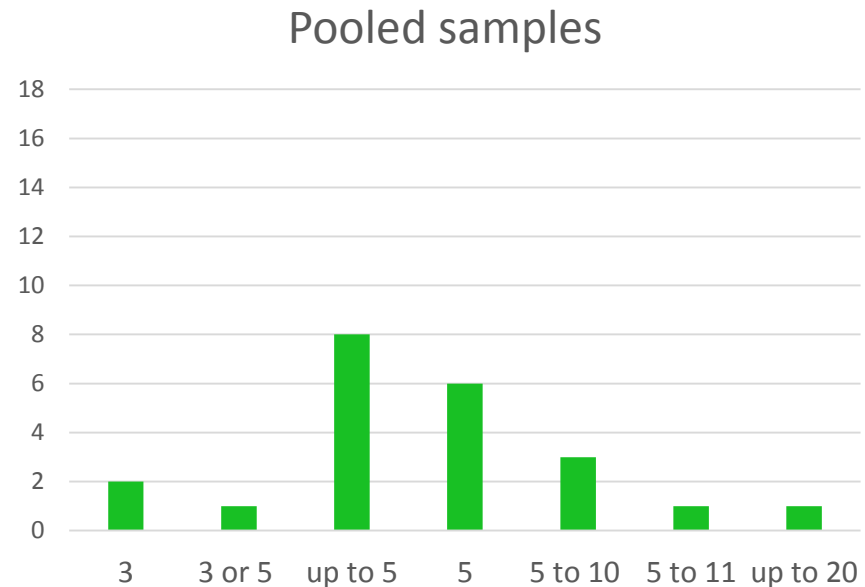
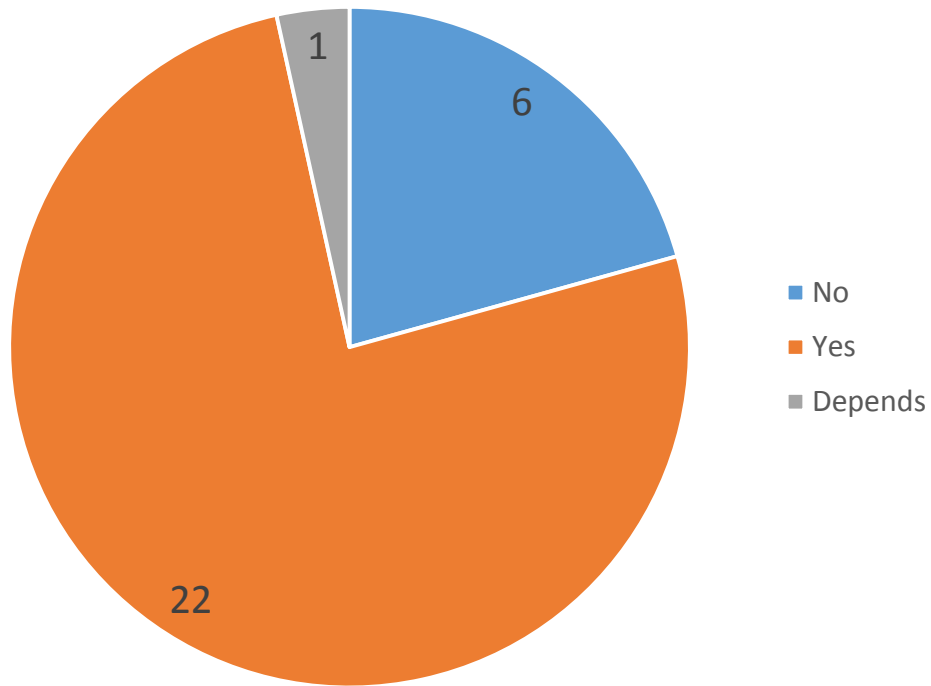
AI must be always considered among the cause of suspected episodes of ND with, for example, high mortality, respiratory signs, eggs drop or nervous syndromes.

AI 1.3 Do you routinely inoculate eggs with samples from disease suspicions in poultry in parallel with molecular testing or after positive molecular tests?



- Most of the NRLs maintain the capacity to isolate AI in eggs but many of them perform VI only after the positive PCR results;
- This can be risky in case of introductions of new variants of AIVs and forces us to control very frequently the performance of the biomolecular methods in use.

AI 1.4 Do you use swab pooling? If yes, please indicate how many samples are pooled into a single pool for testing.



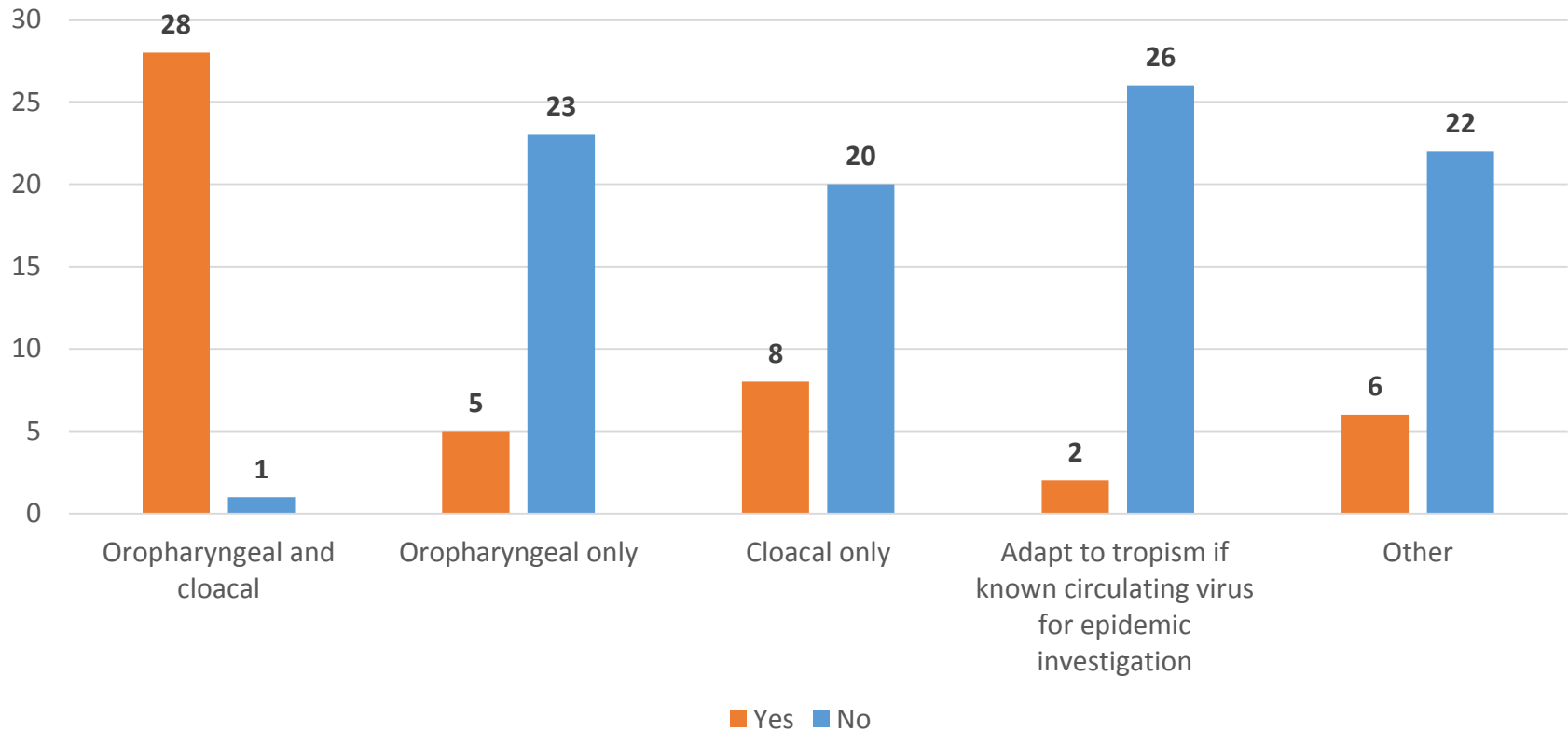
Diagnostic Manual: *Samples may be pooled in batches of five provided they are derived from the same species, time and epidemiological unit.*

● Diagnostic Manual (Commission Decision of 4 August 2006)

- *At least 20 tracheal/oropharyngeal and 20 cloacal swabs.*
- *Birds showing clinical disease signs must be targeted for sampling.*
- *The competent authority may decide to choose either tracheal/oropharyngeal or cloacal swabs rather than to collect both depending on whether the virus replicates better in the respiratory or gastrointestinal tract and also taking into account the species concerned.*
- *Where the species of poultry or other captive birds are not expected to clearly express clinical signs of disease, the competent authority may decide,that the standard samples must be taken from each production unit.*
- *Based on the outcome of a risk assessment, the competent authority must decide sampling for laboratory tests in targeted holdings, compartments or production types.*

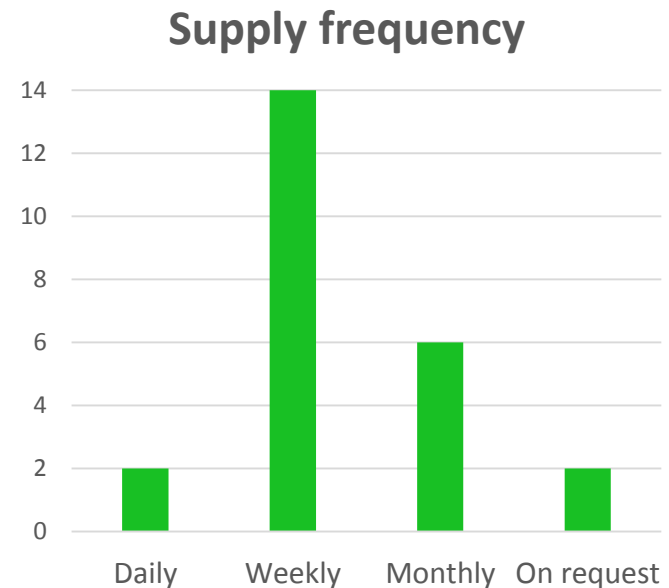
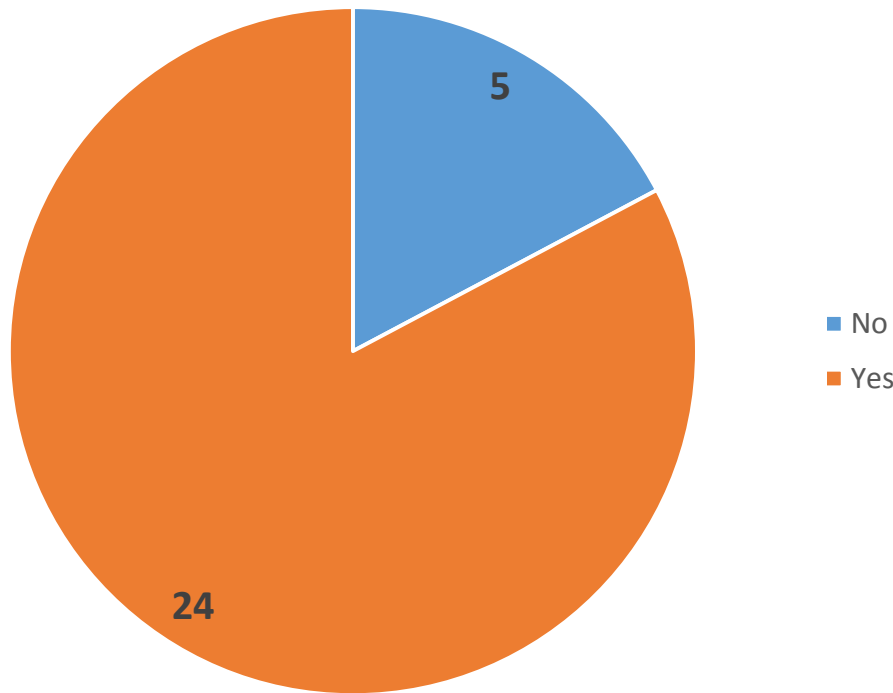
This means that in some circumstances we can collect more than 200 samples from one holding and from numerous holdings every day with the need to adequately pool the samples to process them in good time

AI 1.8. Which type of swabs are generally submitted to the lab and tested?



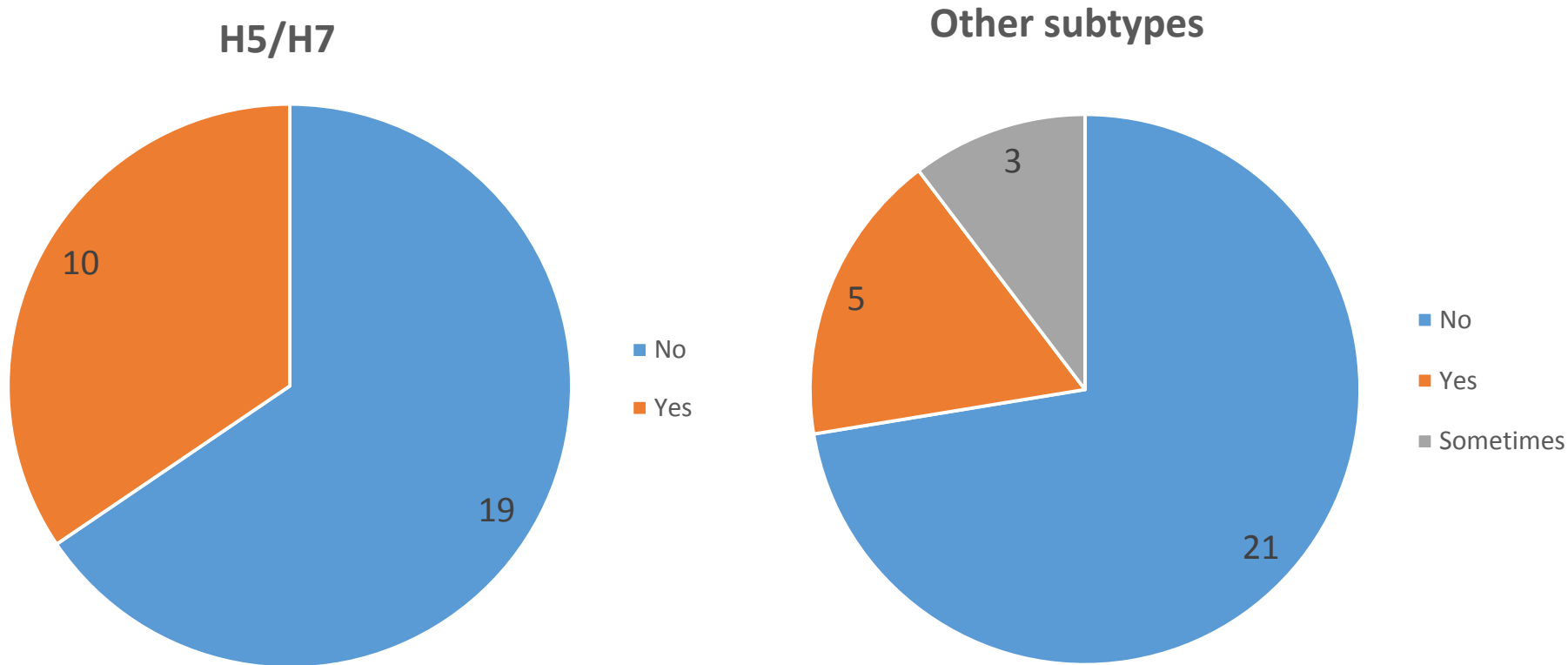
Collecting the most appropriate swabs based on the tropism of the virus, which would represent a more economical and efficient approach, seems to be not a very common practice

AI 1.5. Do you have access to either embryonated SPF or SAN fowls' eggs? If yes, what is the frequency of supply?



Some laboratories may have lost the ability to perform VI in eggs or find difficult to access embryonated eggs

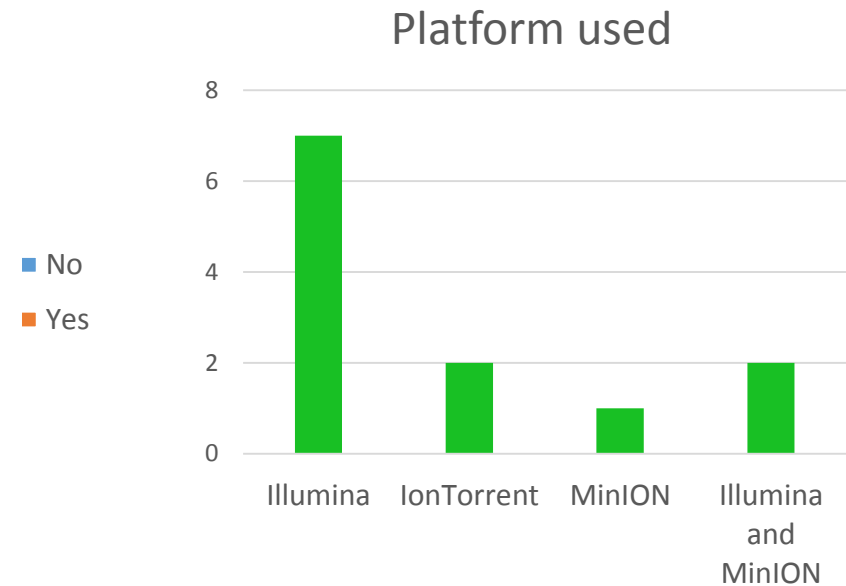
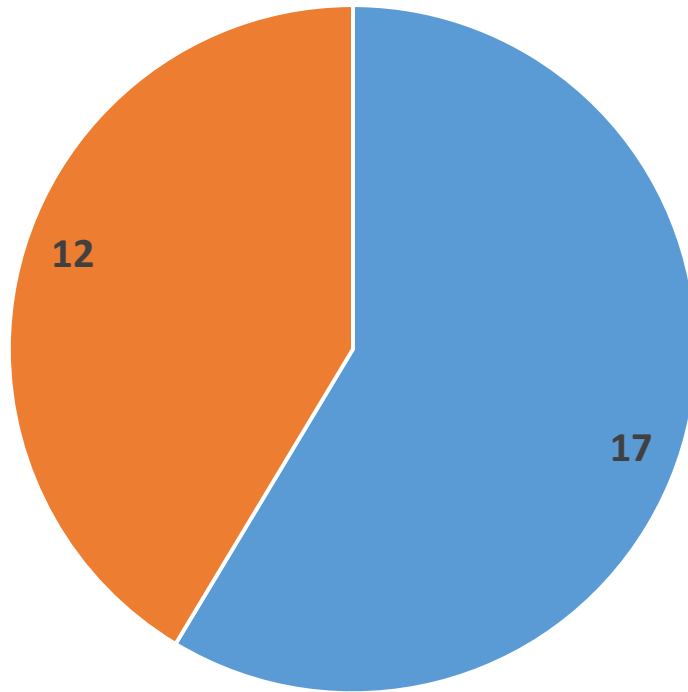
AI 1.6. Do you perform the IVPI test on H5 and H7 isolates? Do you perform the IVPI test also on other subtypes (e.g. H10)?



Abandoning IVPI tests could be contestable, because even if the use of molecular methods for the pathotyping of AIVs is highly appropriate, it is not always able to correctly identify the biological characteristics of emerging viruses

Diagnostic Manual: *The virulence for chickens of influenza A viruses isolated from birds must be estimated using the intravenous pathogenicity index (IVPI) test*

AI 1.7. Do you use Next Generation Sequencing for influenza type A detection and/or characterization?
If yes, please indicate used platform.

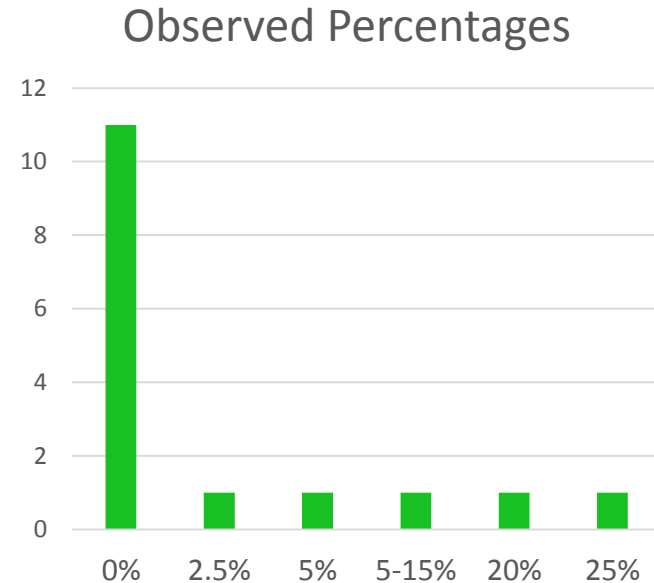
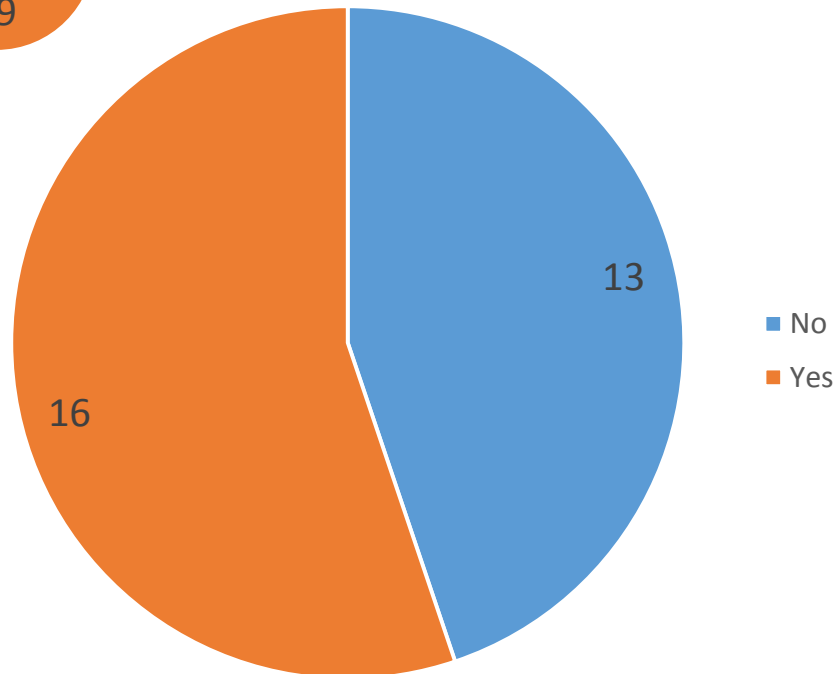


New technologies that allow the sequencing of large portions of the genome in a limited time are used in some laboratories, but are not very widespread yet

AI 2.3. Do you know the approximate percentage of positive results obtained by PCR or virus isolation after collection of samples in response to sero-positive flocks for H5 or H7?

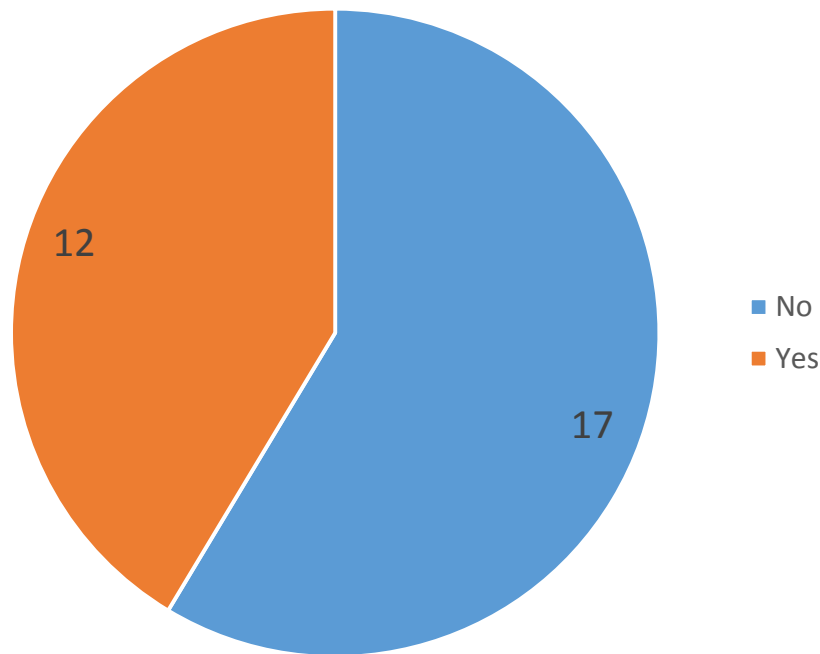


100% of laboratories perform investigations in response to the detection of sero-positive flocks for H5 or H7 under formal EU surveillance programmes



In most cases the detection of seropositivity occurs when the responsible virus is no longer circulating: need to plan more efficient surveillance activities

AI 2.4. Do you conduct any diagnostic tests alternative to serological assays when implementing mandatory surveillance activities for AI?



Alternative tests	Number
M-gene PCR	5
M-gene PCR on duck samples	3
M-gene PCR on game bird offspring	1
Sampling in sentinel mallard station	1
AGID tests and M-gene PCR	1
VI on OPH-S and C-S at broiler import	1

Most laboratories perform only surveillance based on serological methods and this can reduce the possibility of identifying/isolating the circulating viruses (remember also the previous slide)



Summary of results of diagnostic laboratory tests

● Avian Influenza virus-positive samples in 2018 per MS

MS(s)	RT-PCR positive samples
Austria	7
Belgium	71
Bulgaria	449
Croatia	35
Denmark	73
Estonia	9
Finland	4
France	132
Germany	181
Hungary	30
Ireland	4
Italy	17
The Netherlands	94
Slovakia	4
Slovenia	1
Spain	4
Sweden	16
Switzerland	2
United Kingdom	131
Total	1264

Laboratories with **no influenza viruses** isolated or detected

- Cyprus
- Czech Republic
- Greece
- Latvia
- Luxembourg
- Norway
- Poland
- Portugal
- Romania
- Slovenia

2018 vs 2017: 10 vs 8

HPAI H5N6 viruses isolated/detected in 2018

MS(s)	Bird species	Number of detections	Amino Acids at the HA0 cleavage site	Availability of virus isolate
United Kingdom	Duck (Wild)	30	PLREKRRKR/GLF	Yes
United Kingdom	Goose	10	PLREKRRKR/GLF	Yes
United Kingdom	Mute Swan	67	PLREKRRKR/GLF	Yes
United Kingdom	Gull sp.	9	PLREKRRKR/GLF	Yes
United Kingdom	Raptors	9	PLREKRRKR/GLF	Yes
United Kingdom	Unspecified	5	PLREKRRKR/GLF	Yes
Finland	White-tailed eagle	3	PLREKRRKR/GLF	yes
Germany	Chicken	15	PLREKRRKR/GLF	Yes
Germany	Duck	20	PLREKRRKR/GLF	Yes
Ireland	White-tailed eagle	2	PLREKRRKR/GLF	Yes
Ireland	Common buzzard	1	PLREKRRKR/GLF	Yes
The Netherlands	Chicken	40	PLREKRRKR/GLF	Yes
The Netherlands	Duck	40	PLREKRRKR/GLF	Yes
The Netherlands	Captive birds	14	PLREKRRKR/GLF	Yes
The Netherlands	Wild birds	2	PLREKRRKR/GLF	Yes
Slovakia	Black headed gull	3	PLREKRRKR/GLF	No
Sweden	Chicken	1	PLREKRRKR/GLF	Yes
Sweden	White-tailed eagle	13	NA	Yes
Sweden	Northern goshawk	1	NA	Yes
Sweden	Common buzzard	2	NA	Yes

HPAI H5N6 viruses isolated/detected in 2018

MS(s)	Bird species	Number of detections	Amino Acids at the HA0 cleavage site	Availability of virus isolate
Denmark	Common eider	2	PLREKRRKR/GLF	No
Denmark	Pheasant	5	PLREKRRKR/GLF	No
Denmark	Mallard	2	PLREKRRKR/GLF	No
Denmark	Greylag goose	1	PLREKRRKR/GLF	No
Denmark	Hooded crow	2	PLREKRRKR/GLF	No
Denmark	White-tailed eagle	12	PLREKRRKR/GLF	Yes
Denmark	Black-headed gull	1	PLREKRRKR/GLF	No
Denmark	Mute swan	4	PLREKRRKR/GLF	No
Denmark	Common buzzard	11	PLREKRRKR/GLF	No
Denmark	Great cormorant	1	PLREKRRKR/GLF	No
Denmark	European herring gull	1	PLREKRRKR/GLF	No

HPAI H5N8 viruses isolated/detected in 2018

MS(s)	Bird species	Number of detections	Amino Acids at the HA0 cleavage site	Availability of virus isolate (Yes/No)
Bulgaria	Mule ducks	352	PLREKRRKR/GLF	Yes
Bulgaria	Chicken (hens)	62	PLREKRRKR/GLF	Yes
Bulgaria	Chicken (broilers)	32	PLREKRRKR/GLF	Yes
Bulgaria	Turkey	2	PLREKRRKR/GLF	Yes
Italy	Turkey	3	PLREKRRKR/GLF	Yes
Italy	Chicken (hens)	2	PLREKRRKR/GLF	Yes

● Summary - HPAI situation in 2018

- 7 MSs reported isolation/detection of H5N6 (compared to 1 MS in 2017)
- 2 MSs reported isolation/detection of H5N8 (compared to 19 MSs in 2017)
- 1 MS (UK) reported isolation/detection of H5N6 and H5N8 subtype in 2018 but the latter was a LPAIV
- No other HPAI subtypes detected (limited reassortment with other subtypes?)
- Cleavage site was the same for all the HPAI isolates sequenced in 2018: PLREKRRKR/GLF (Offlu document still actual)

H5 LPAI viruses isolated/detected in 2018

LPAI Subtype	Number of detection(s)	MS(s)
H5N*	44	
Duck	6	Denmark (3), France (3)
Mallard	37	Belgium
Wild Bird (Unspecified)	1	Denmark (1)
H5N1	3	
Duck	1	France (1)
Turkey	1	France (1)
H5N2	10	
Captive Mallard	1	France (1)
Duck	6	France (6)
Farmed mallards	1	Denmark (1)
Mute Swan	1	Slovenia (1)
Mallard	1	Italy (1)
H5N3	28	
Captive birds	16	The Netherlands (16)
Duck	12	France (11), Italy (1)
H5N5	2	
Duck	1	France (1)
Duck (Wild)	1	Austria (1)
H5N7	2	
Duck	2	France (1), Italy (1)
H5N8	1	
Swan	1	United Kingdom
Total	90	

H7 LPAI viruses isolated/detected in 2018

LPAI Subtype	Number of detection(s)	MS(s)
H7N3	3	
Wild duck	3	Italy
H7N7	2	
Captive mallard	1	France
Wild mallard	1	Italy
Total	5	

● Non H5-H7 LPAI viruses isolated/detected in 2018

LPAI Subtype	Number of detection(s)	LPAI Subtype	Number of detection(s)
H*N*	162	H6N1	15
H*N1	2	H6N2	5
H*N2	1	H6N8	5
H*N3	3	H8N2	2
H*N5	1	H8N4	1
H1N*	2	H9N*	2
H1N1	7	H9N2	140
H1N3	1	H9N3	2
H1N8	22	H9N7	10
H2N*	1	H9N8	1
H2N2	1	H10N1	1
H2N3	1	H10N3	1
H3N2	1	H11N*	1
H3N8	6	H11N9	4
H4N*	1	H12N5	1
H4N2	2	H13N2	1
H4N6	5	H13N8	1
H6N*	27		

Total= 440

H1 LPAI viruses isolated/detected in 2018

LPAI Subtype	Species	Number of detection(s)	MS(s)
H1N*	Ducks	2	France
H1N1	Ducks	1	France
	Mulard	2	Hungary
	Mallard	4	Belgium
H1N3	Farmed mallards	1	Denmark
H1N8	Eurasian coot	1	Denmark
	Mulard	20	Hungary
	Duck	1	Hungary
Total		32	

H2 LPAI viruses isolated/detected in 2018

LPAI Subtype	Species	Number of detection(s)	MS(s)
H2N*	Ducks	1	France
H2N2	Chicken	1	France
H2N3	Mallard	1	Belgium
Total		3	

H3 LPAI viruses isolated/detected in 2018

LPAI Subtype	Species	Number of detection(s)	MS(s)
H3N2	Common teal	1	Denmark
H3N8	Mallard	1	Belgium
	Common teal	2	Denmark
	Duck	1	France
	Wild duck	1	Spain
	Wild mallard	1	Italy
	Wild bird (Unspecified)	1	Netherlands
Total		8	

H4 LPAI viruses isolated/detected in 2018

LPAI Subtype	Species	Number of detection(s)	MS(s)
H4N*	Duck	1	France
H4N2	Mallard	1	Belgium
	Mule ducks	1	Bulgaria
H4N6	Mallard	3	Belgium
	Domestic duck	1	Italy
	Wild mallard	1	Italy
Total		8	

H6 LPAI viruses isolated/detected in 2018

LPAI Subtype	Species	Number of detection(s)	MS(s)
H6N*	Common murre[^]	2	Denmark
	Duck	13	France
	Chicken	10	Netherlands
	Cormorant	1	Hungary
	Mallard	1	Hungary
H6N1	Duck	11	France
	Turkey	1	France
	Goose	2	Hungary
	Common teal	1	Italy
H6N2	Common murre[^]	2	Denmark
	Duck	1	France
	Turkey	1	France
	Great White Egret	1	Hungary
H6N8	Duck	3	France
	Goose	1	Hungary
	Mallard	1	Hungary
Total		52	

[^]Birds found dead in Greenland.

H8 LPAI viruses isolated/detected in 2018

LPAI Subtype	Species	Number of detection(s)	MS(s)
H8N2	Common teal	1	Denmark
	Northern shoveler	1	Denmark
H8N4	Wild bird (Unspecified)	1	The Netherlands
Total		3	

H9 LPAI viruses isolated/detected in 2018

LPAI Subtype	Species	Number of detection(s)	MS(s)
H9N*	Duck	2	France
H9N2	Turkey	140	Germany
H9N3	Turkey	2	Germany
H9N7	Duck	10	France
H9N8	Turkey	1	Germany
Total		155	

● H10/11/12/13 LPAI viruses isolated/detected in 2018

LPAI Subtype	Species	Number of detection(s)	MS(s)
H10N1	Mallard	1	Belgium
H10N3	Mute swan	1	Switzerland
H11N*	Wild mallard	1	Estonia
H11N9	Common teal	1	Denmark
	Nandu	3	Germany
H12N5	Wild mallard	1	Estonia
H13N2	Black-headed gull	1	Croatia
	Captive mallard	1	Slovakia
H13N8	Yellow-legged gull	1	Croatia
Total		10	

All H subtypes detected with the exception of H14, H15 and H16
 All N subtypes detected

Wild Bird species with H5/H7 - RT-PCR positive results

Species	Number of RT-PCR positive samples
Black-headed gull	1
Gull sp.	9
Great cormorant	1
European herring	1
Mute Swan	73
Northern goshawk	1
Common buzzard	14
Raptors	9
White-tailed eagle	30
Duck (Wild)	31
Mallard (Wild)	4
Pheasant	5
Common eider	2
Hooded Crow	2
Greylag goose	1
Wild Bird (Unspecified)	13
Total	197

Wild Bird species with **NON-H5/H7** - RT-PCR positive results

Species	Number of RT-PCR positive samples
Wild Bird (Unspecified)	53
Wild Duck	7
Common teal	5
Mute Swan	5
Common murre	4
Nandu (Zoo)	3
Wild mallard	3
Barnacle goose	2
Eurasian wigeon	2
Black-headed gull	1
Eurasian coot	1
Long-tailed duck	1
Northern pintail	1
Northern shoveler	1
Yellow-legged gull	1
Total	90

● SUBMISSION OF VIRUSES TO EURL

- Thank to the NRLs which have submitted viruses
- A reminder: EU AI diagnostic manual (2006/437/EC) Chapter V; Section 4: *The Community reference laboratory must receive all haemagglutinating viruses from the National reference laboratories for further antigenic and genetic studies*
- Timely submission H5/H7 (primary at least)
- All HA agents/primary detections
- Vital to evaluate diagnostic methods (real time and conventional PCR) and reference reagents
- Production of antigens to be used in sero-survey
- Reagent production (sera for classical typisation)

**THANK YOU FOR YOUR ATTENTION
AND
FOR RETURNING YOUR QUESTIONNAIRES**