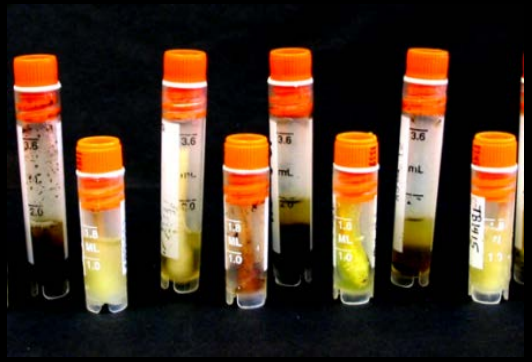
IMPROVEMENTS IN AIV SPECIMEN COLLECTION, TRANSPORT AND PROCESSING



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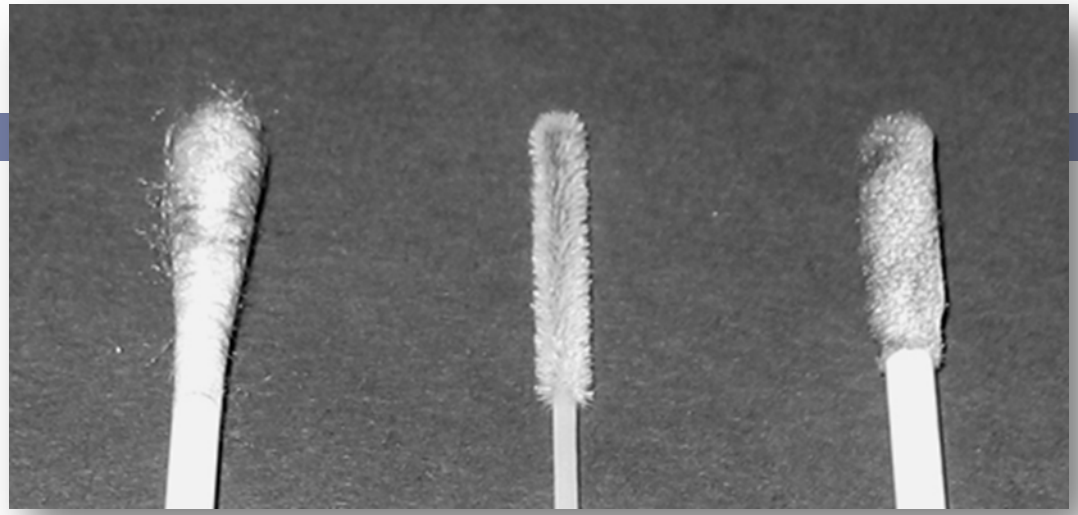
Swab Type



- Public health research has shown differences in respiratory virus detection among swab types
  - Capture and release
- Current swab most commonly used in the US
  - Non-flocked/wound (dacron/nylon tipped), plastic shaft
- Newer types
  - Flocked (nylon)
  - Foam (urethane)
- All cost the same (~$0.10 USD)
- All have the same head size

Media

- Brain-heart infusion broth (BHI) and phosphate buffered saline (PBS) have been evaluated for virus isolation (Erickson, 1976)
  - Does it matter for rRT-PCR or antigen detection tests?
    - BHI expensive and has a relatively short shelf life
- Numerous other media available: BHI and PBS common and widely used.
  - Represent protein vs. non-protein broths

Inoculate $10^{6.5}EID_{50}$ of A/turkey/VA/SEP-67/2002 H7N2 by the intra-choanal route

Terminated

The first experiment included 14, 17 and 21 DPI, but all were negative

Days PI when swabs were collected

0 1 2 3 4 7 10

Each bird only swabbed once per day

Pools always consisted of one swab from an inoculated chickens and the rest from uninfected chickens

n=15 OP swabs or pools per day
n=15 CL swabs or pools per day

Media: virus isolation, OP swabs

BHI Best

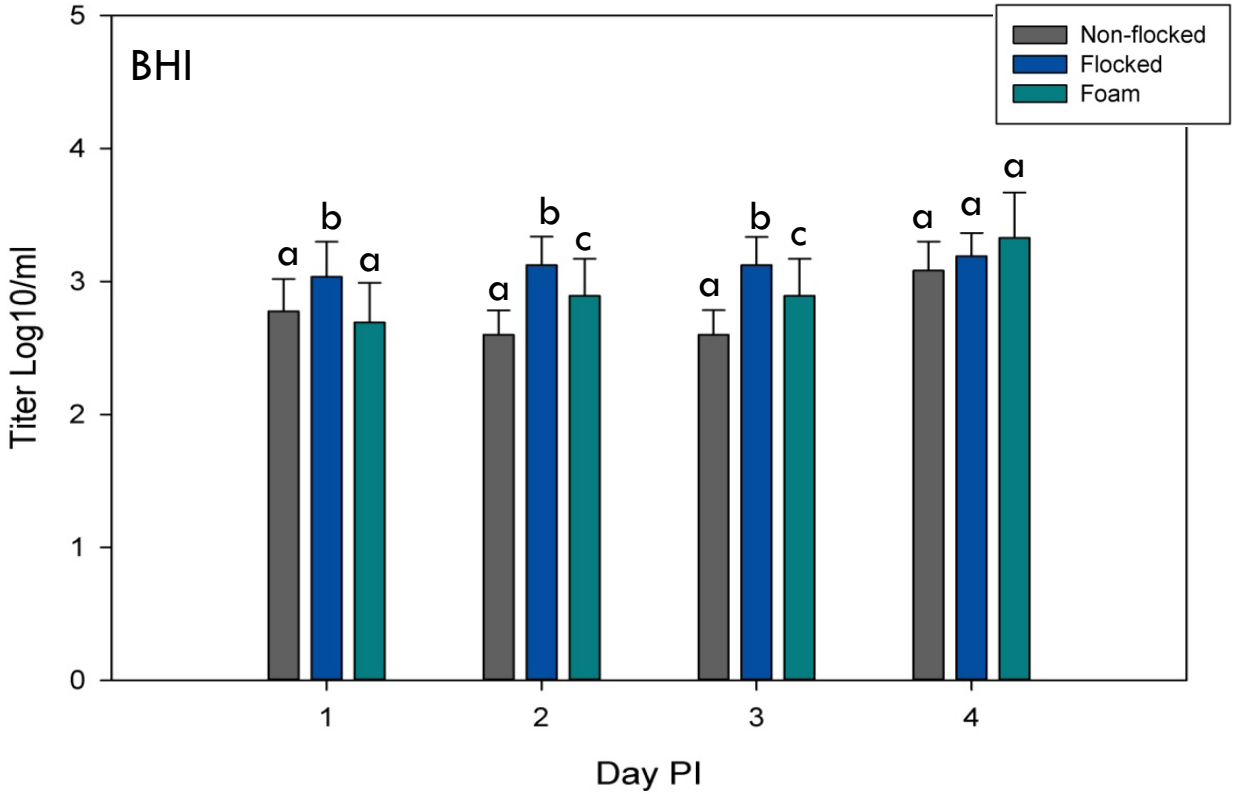
PBS Poor virus recovery

	BHI				PBS		
	2 DPI	3 DPI	4 DPI		2 DPI	3 DPI	4 DPI
Non-flocked	100	100	100		30	70	0
Flocked	100	100	100		50	80	0
Foam	100	100	90		60	90	0

Swab type: rRT-PCR OP Swab Titers

1, 2, 3, 4 DPI- 100% positive

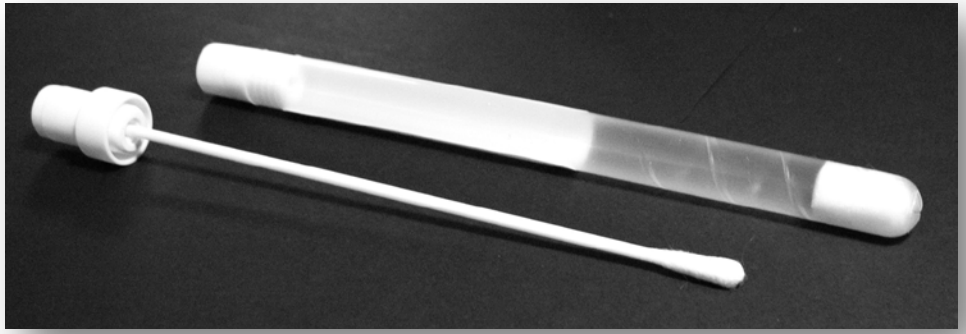
10 and 14 DPI- All negative



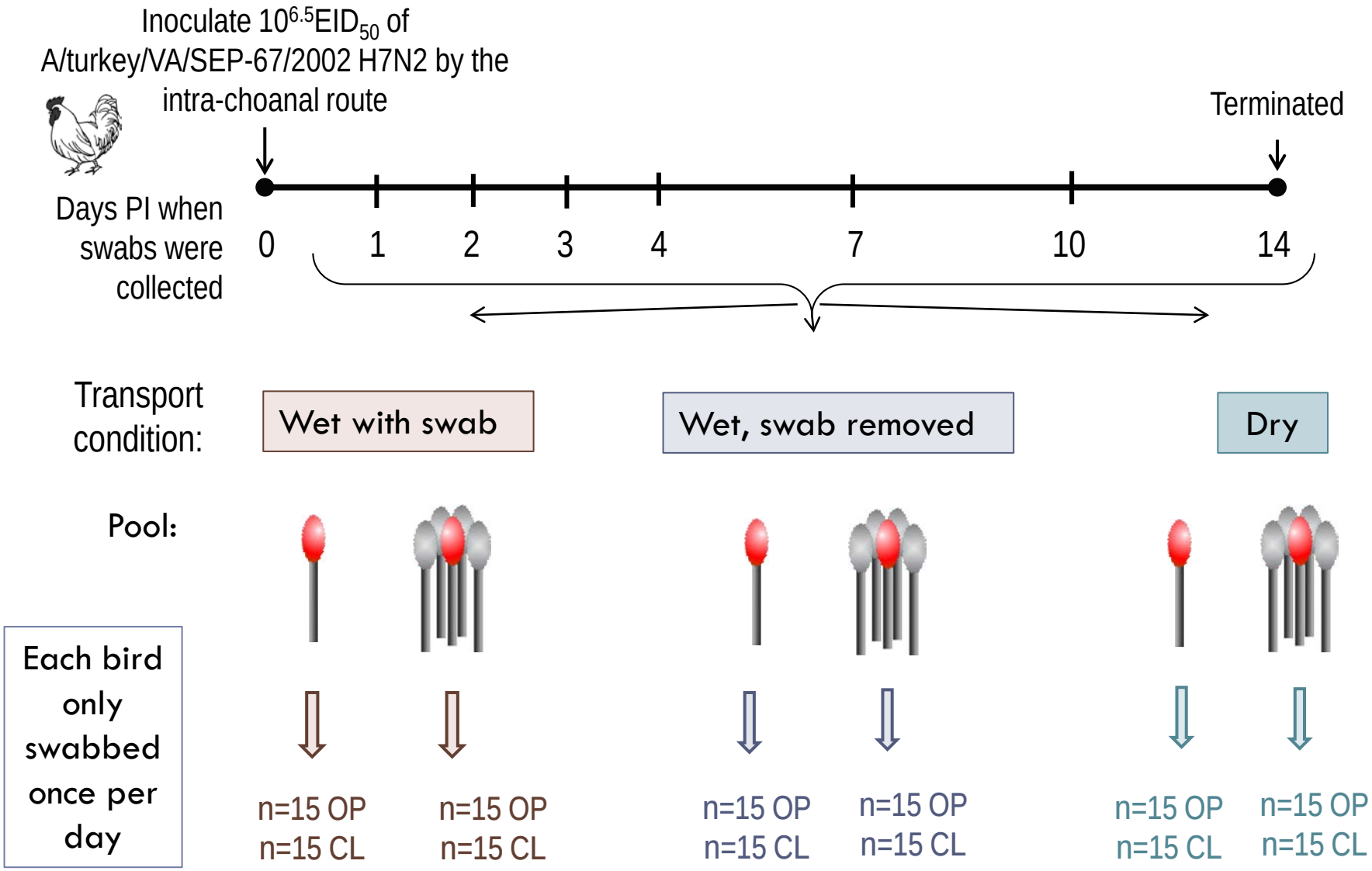
Flocked swabs superior to non-flocked swabs

Swab transport

- Wet vs dry
- Swab removed vs left in during transport
  - Must express all liquid when removing the swab
- All with BHI and flocked swabs

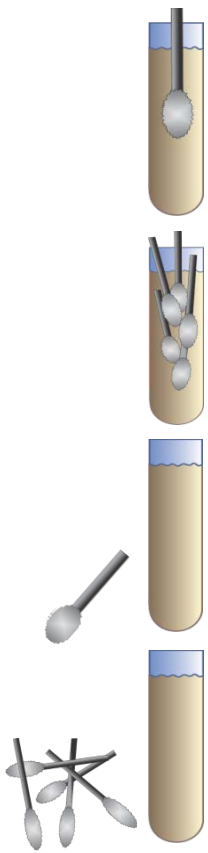


Culturette used for dry swab transport


Inoculate $10^{6.5}EID_{50}$ of A/turkey/VA/SEP-67/2002 H7N2 by the intra-choanal route
Terminated
Days PI when swabs were collected
0
1
2
3
4
7
10
14
Transport condition:
Wet with swab
Wet, swab removed
Dry
Pool:
Each bird only swabbed once per day
n=15 OP
n=15 CL
n=15 OP
n=15 CL
n=15 OP
n=15 CL
n=15 OP
n=15 CL
n=15 OP
n=15 CL
n=15 OP
n=15 CL

Percent positive: To leave the swab in or not to leave the swab in?

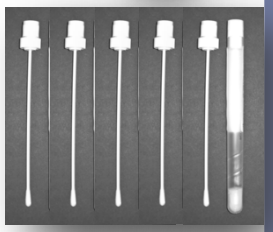
No observable difference



Swab removed	# per vial	2 DPI		3DPI		4 DPI		7 DPI	
		VI	PCR	VI	PCR	VI	PCR	VI	PCR
No	1	100	100	100	100	100	100	20	13.3
No	5	100	100	100	100	100	100	20	0
Yes	1	100	100	90	93.3	100	100	40	0
Yes	5	100	93.3	100	100	100	100	20	0

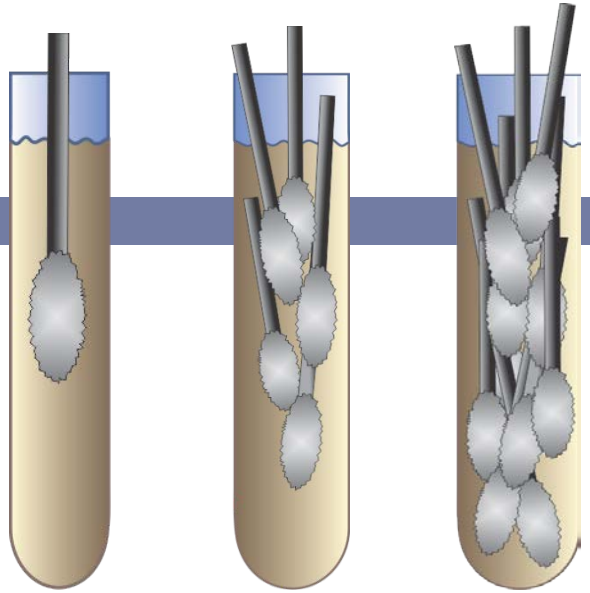
Percent positive: Dry transport vs wet

Dry transport reduced virus isolation rates and titers detected by rRT-PCR



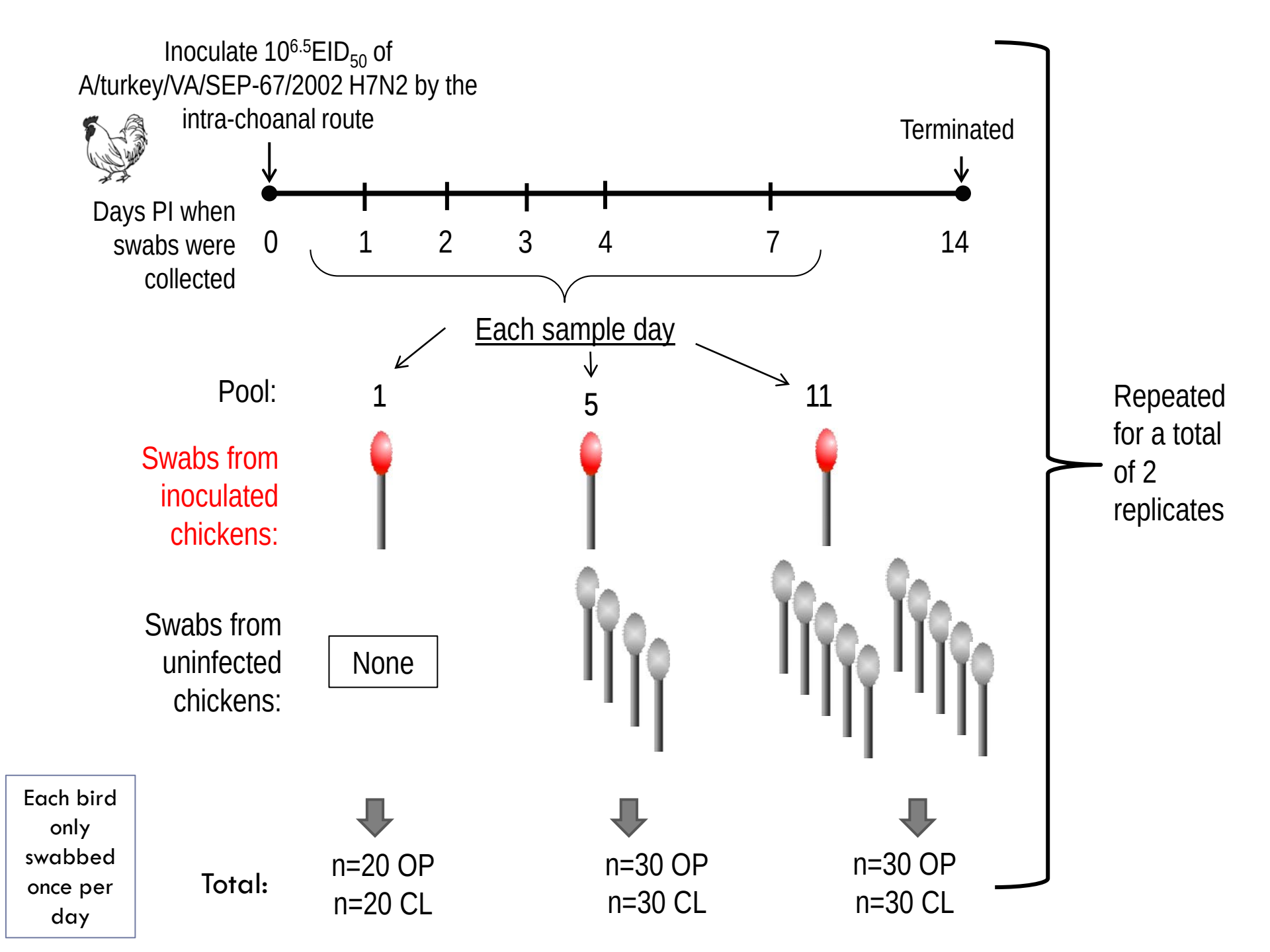
Media	# per vial	2 DPI		3DPI		4 DPI		7 DPI	
		VI	PCR	VI	PCR	VI	PCR	VI	PCR
3.5 ml BHI	1	100	100	100	100	100	100	20	13.3
3.5ml BHI	5	100	100	100	100	100	100	20	0
None (dry)	1	80	80	60	100	70	93.3	0	0
None (dry)	5	100	93.3	40	93.3	60	93.3	0	0

Swab Pooling



- 1 vs. 5 vs. 11 per vial
  - Virus Isolation
  - rRT-PCR

- Newcastle disease virus evaluated by Jan Pedersen at the US National Veterinary Services Laboratories using the same procedure with a mesogen (Roakin strain).


Inoculate $10^{6.5}EID_{50}$ of A/turkey/VA/SEP-67/2002 H7N2 by the intra-choanal route
Terminated
Days PI when swabs were collected
0
1
2
3
4
7
14
Each sample day
Pool:
1
5
11
Swabs from inoculated chickens:
Swabs from uninfected chickens:
None
Total:
n=20 OP
n=20 CL
n=30 OP
n=30 CL
n=30 OP
n=30 CL
Repeated for a total of 2 replicates
Each bird only swabbed once per day

Swab pooling:
Percent of OP swabs positive for AIV

Swab pool	1DPI		2DPI		3DPI		4DPI		7DPI	
	VI	PCR	VI	PCR	VI	PCR	VI	PCR	VI	PCR
1	100	100	80	95	100	100	100	100	10	80
5	100	93.3	100	96.6	100	100	100	100	30	86.6
11	95	96.6	95	100	100	100	100	100	15	83.3

No difference in detection based on the numbers of swabs per tube.

Swab pooling:
Percent of OP swabs positive for NDV

Swab pool	1DPI		2DPI		3DPI		4DPI		7DPI	
	VI	PCR	VI	PCR	VI	PCR	VI	PCR	VI	PCR
1	100	100	100	100	100	100	100	100	5	70
5	100	100	100	100	100	100	100	100	2.6	68.4
11	100	100	100	100	100	100	100	100	5.3	36.8

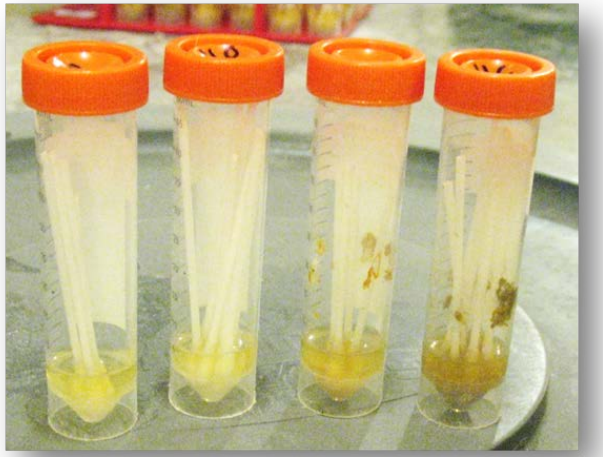
No difference in detection based on the numbers of swabs per tube.

Swab Pooling

- AIV
  - No difference in the number positive by virus isolation or rRT-PCR
  - Too few CL swabs were positive to draw conclusions
- NDV
  - No difference in the number positive by virus isolation or rRT-PCR
- **Pooling up to 11 OP swabs did not affect rRT-PCR or virus isolation**

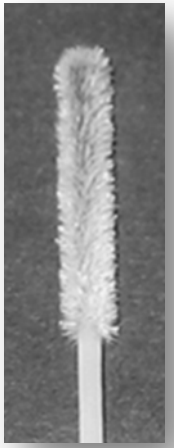
Swab pooling

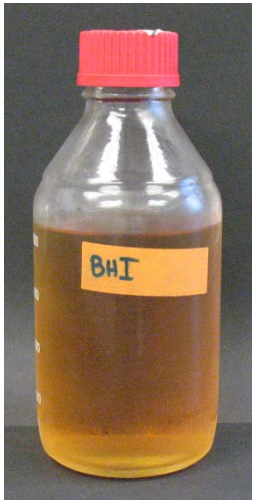
- Other considerations
  - Pools of 11 require a minimum of 5.5ml media
  - Require larger tubes
  - Puts all you swabs in one tube; no back-up if loss or breakage occurs
  - Impacts work flow/logistics at the lab, particularly if the swabs are left in

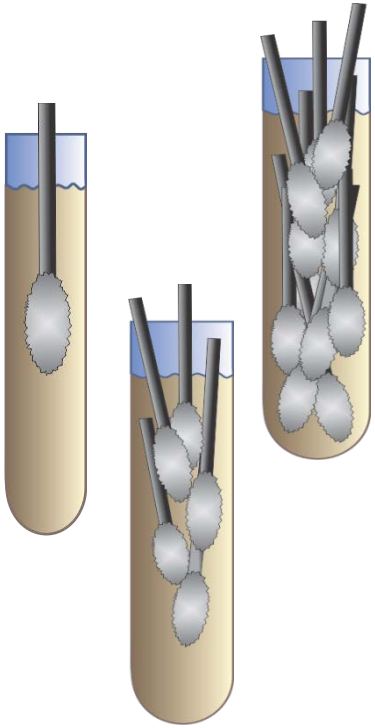


Recommendations

1. Flocked swabs
2. BHI
3. Up to 11 swabs
4. Transport with swab in or out







Recommendations

1. Do not use PBS
2. Do not transport swabs without media

Acknowledgements

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- Anna Linz
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