

# The Molecular Diagnosis of EIA, a Permanent Challenge or an Attainable Goal?

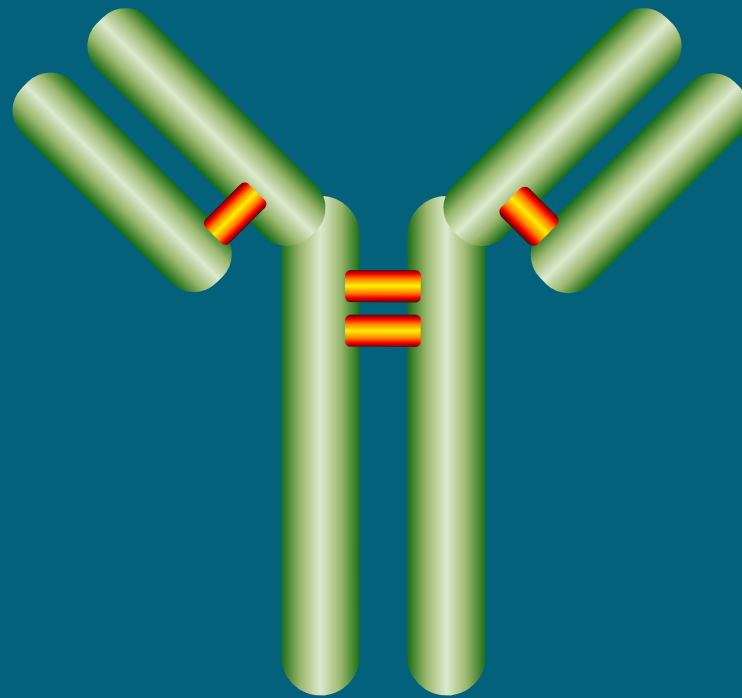


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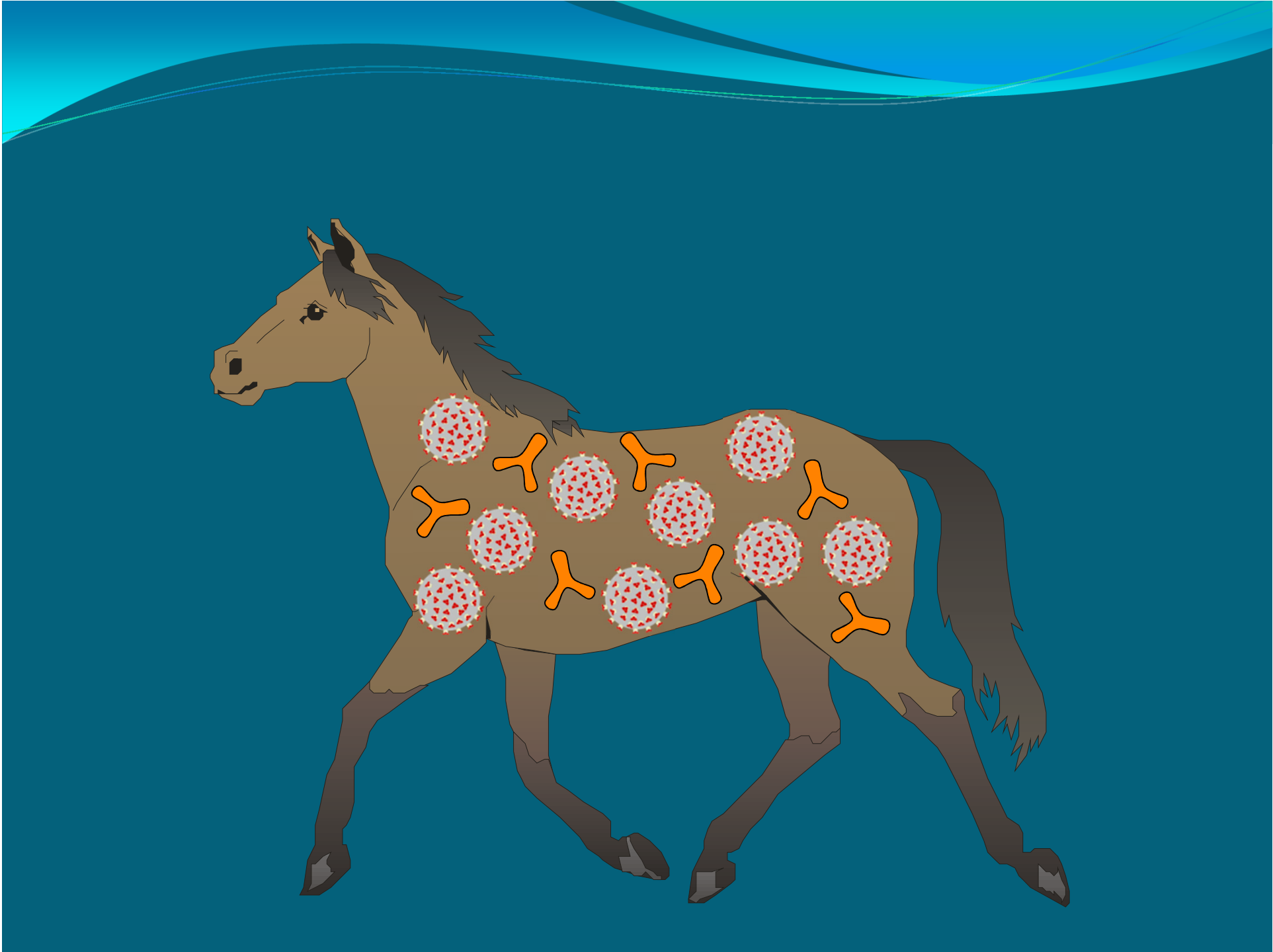


# **Why is There a Need for Molecular Detection of EIAV?**

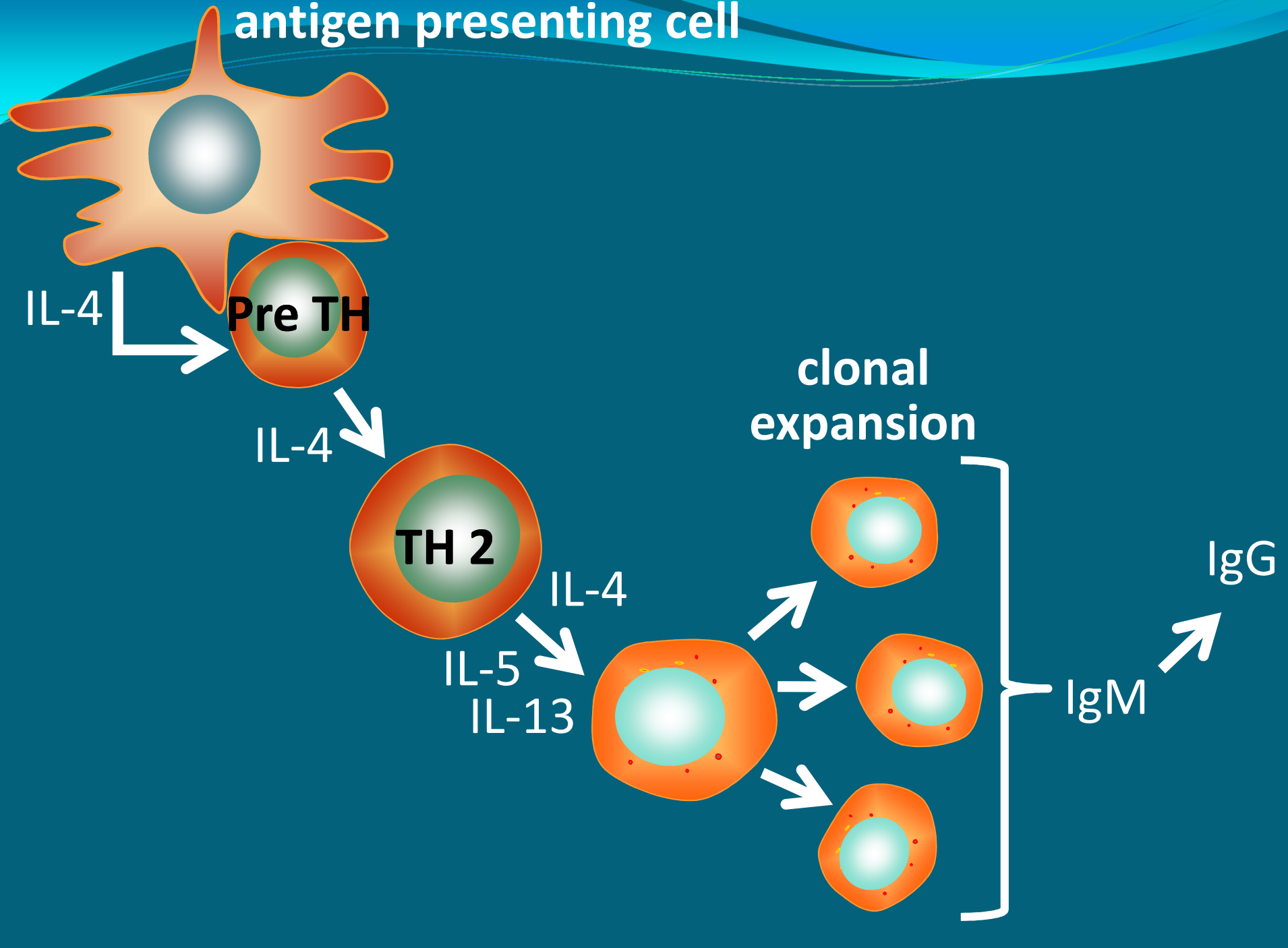
# EIAV: Serological Diagnosis



Indirect Method of Detection



antigen presenting cell



# EIA

- Indirect Detection Disadvantages:

- ❖ Incubation Period (AGID)

- Most cases within 45 days (Issel & Cook 1993)
    - Longest reported 157 days (Cullinane et. al. 2007)

- ❖ Italy 2006: Farm

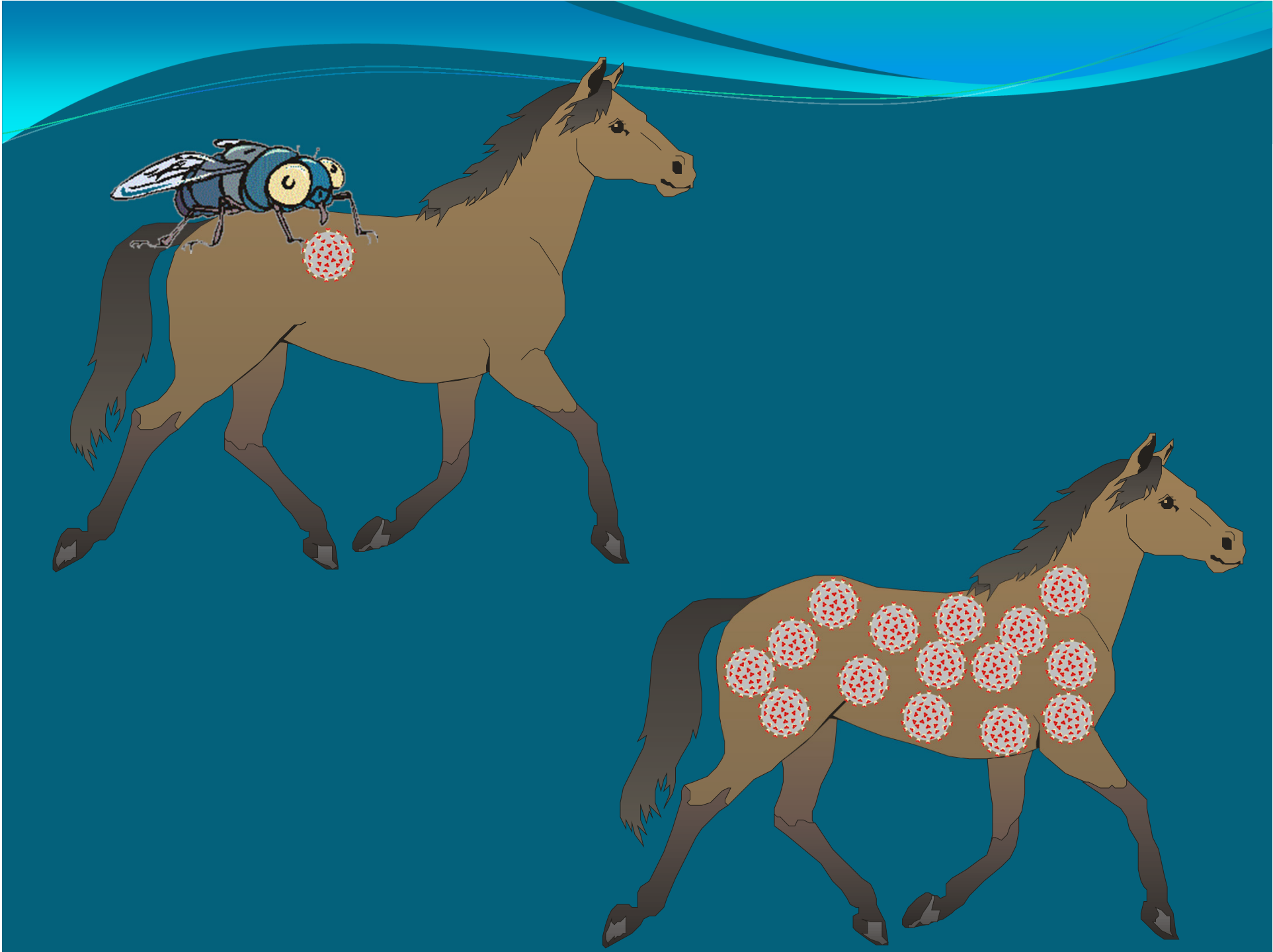
- 90 day quarantine : ALL AGID –ve
    - 5 months later: 8 NEW cases

- ❖ Argentina 2012: Riding School

- Horse with –ve AGID

# EIAV : A Need for Direct Detection

Recent Exposure





# EIAV: Direct Detection Methods

Virus Isolation:

Equine MØ

Insensitive

Highly Variable

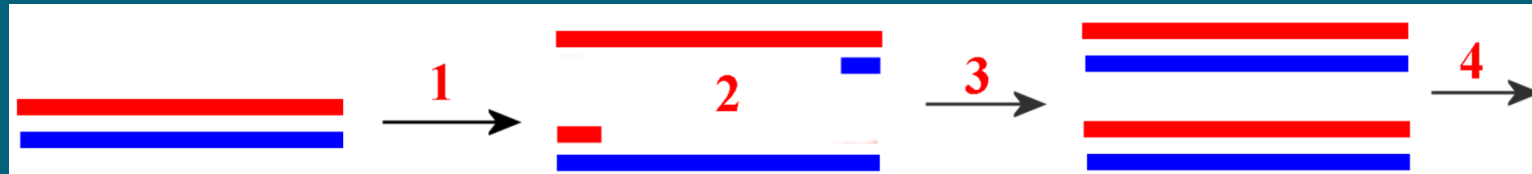
Protein: Sensitivity

Nucleic Acid: vRNA/Proviral DNA

PCR

Isothermal

# PCR



**1: Denaturation (95°C)**  
**2: Anneal (45-60°C)**  
**3: Elongation (65-75°C)**  
**4: "Repeat 1-3"**

Cycle #	# DNA Molecules
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1024

# EIAV PCR: Problems

- PCR sensitive to variation in primer/probe binding sites
- EIAV: HIGH Variability
- Inapparent Carrier: LOW viral loads in blood  
Undetectable viral RNA

# Variation Between EIAV Isolates

Ire	CTGTAAAGAAAGGGCCTACAAATTGGGCATGTAGGC	GATGGAAGGGCAACA		
Ita	CTGTAAAGAAAGGGCCTACAAATTGGGCATGTAGGC	GATGGAAGGGCAACA		
Lia	CCGTTAAGATGGGC	TACAAATTAATAC	TGTGAATGATG	CAAAGCAACA
Can1	CTGTTAAAATGGGCCTC	CAAAATTAATAATGTAGGAGATGGAAGGGCATCG		
Can7	CTGTAAAATGGGCCTC	CAAAATCAATAATGTAGGAGATGGAAGGACATCA		
Can3	CTGTAAAGATGGGCCTC	CAAGTCAGTAATGTAGGAGATGGAAGGCATCA		
Wyo	CTGTAAAGATGGGCCTC	CAGATTAATAATGTAGTAGATGGAAGGCATCA		
Jap	CTGTAAAGATGGGCCTC	CAGATTAATAATGTAGTAGATGGAAGGCATCA		
Can10	CCGTTAAGATGGGCCTC	CAGATTAATCATGTTGGCGATGGTAAAGCATCG		

# PCR Primer Mismatches



Primer TAGGAATTCTCGCCCTTAACG  
Virus ATCCTTAAGAGCGGAATTGC

Primer TAGGAATTCTCGCC  
Virus A CCTTAA T AGCGGAATTGC

annealing temp A/T -2°C G/C -4°C = -6°C

Primer TAGGAATTCTC<sup>GCC</sup>  
Virus ATCCTTAAGAGAAAAATTGC

annealing temp = 3x G/C = -12°C

# EIAV Published PCR Protocols

- Nested
  - ❖ Langemeier et. al. 1996
  - ❖ Nagarajan and Simard, 2001 – OIE
  - ❖ Quinlivan et. al. 2007
  - ❖ Cappelli et. al. 2011
  - ❖ Capomaccio et. al. 2012
  - ❖ Dong et. al. 2012
- Real Time
  - ❖ Cook et.al. 2002

# Retrovirus



Single  
Strand  
RNA

**Viral Genome**

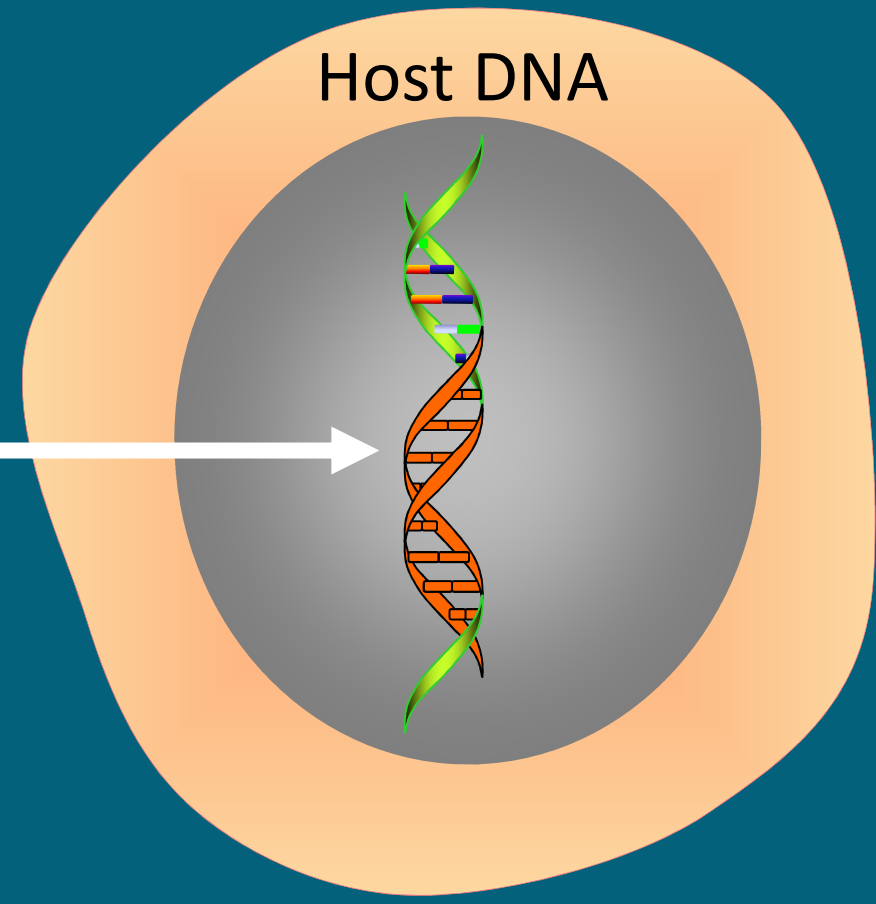
Reverse  
Transcriptase

→



Double  
Stranded  
DNA

**Provirus**



Host DNA

# EIAV: Target Nucleic Acid

- Capelli et. al. 2007

## AGID Comparison

❖ vRNA                      POOR

❖ Proviral DNA      GOOD

- Dong et. al. 2012

❖ Proviral DNA

❖ 12/12 EIA Seropositive Horses



# Comparative PCR Testing

- Nagarajan and Simard, 2001
- Cappelli et. al., 2011
- Dong et. al., 2012
- Modified Cappelli

Samples: Argentina

26 AGID –ve

25 AGID +ve

3 Febrile

Template: 1µg PBMC DNA

TaKaRa Ex Taq™

# Primer Variation

Consensus	1	10	20
	W	B	TGGGGCGYTRARTYTRGTR
1. ITA 1	TG	. . . . .	C . A . G . T . G . . . G
2. ITA 2	TG	. . . . .	C . A . G . T . G . . . G
3. ITA 3	TG	. . . . .	C . A . G . T . G . . . G
4. ITA 5	AT	. . . . .	C . A . G . T . G . . . G
5. IRE	TG	. . . . .	C . A . G . T . G . . . G
6. FL	AC	. . . . .	C . A . G . C . G . . . A
7. NC	AT	. . . . .	C . A . G . T . G . . . A
8. PA	AT	. . . . .	T . A . G . C . G . . . A
9. WY	AT	. . . . .	C . A . G . C . A . . . A
10. Can 1	AT	. . . . .	C . A . G . C . A . . . G
11. Can 3	AT	. . . . .	T . A . G . T . G . . . G
12. Can 7	AC	. . . . .	C . A . A . T . G . . . G
13. Can 10	AT	. . . . .	C . A . G . C . A . . . G
14. Brazil 77	AT	. . . . .	T . A . G . T . G . . . G
15. Brazil 95	AT	. . . . .	T . A . G . T . G . . . G
16. China	AT	. . . . .	C . G . A . T . G . . . G

# PCR Results

	NS	C	D	MC
AGID/Blot -ve p26 (26)	0	0	0	0
AGID/Blot +ve p26 (25)	0	3 (12%)	4 (16%)	4 (16%)
FEBRILE (3)	0	3 (21%)	3 (25%)	3 (25%)

NS: Nagarajan and Simard  
D: Dong et. al.

C: Cappelli et.al.  
MC: Modified Cappelli

# PCR +ve Samples

- Cappelli et. al.      3, 20, 31
- Dong et. al.        3, 8, 20, 31
- Modified            8, 12, 20, 31

