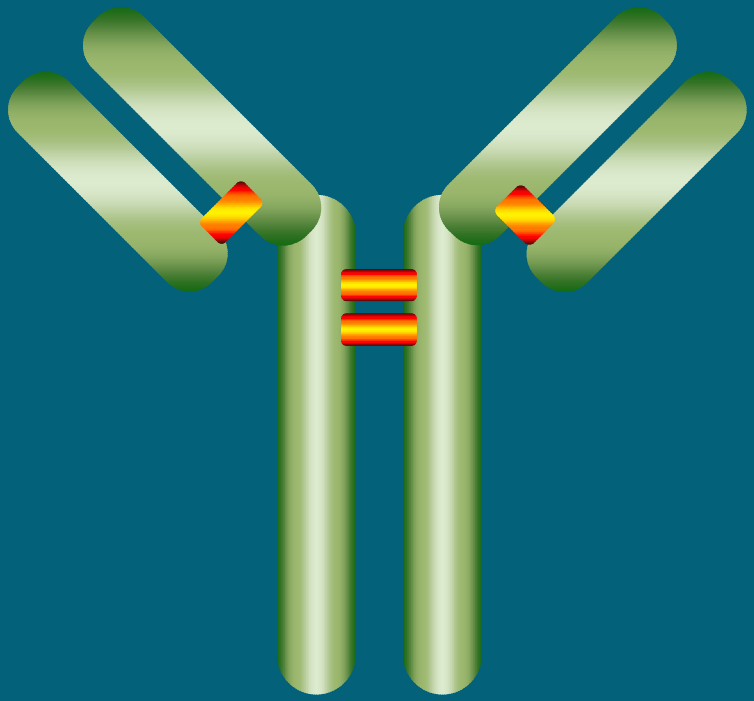
The Molecular Diagnosis of EIA, a Permanent Challenge or an Attainable Goal?

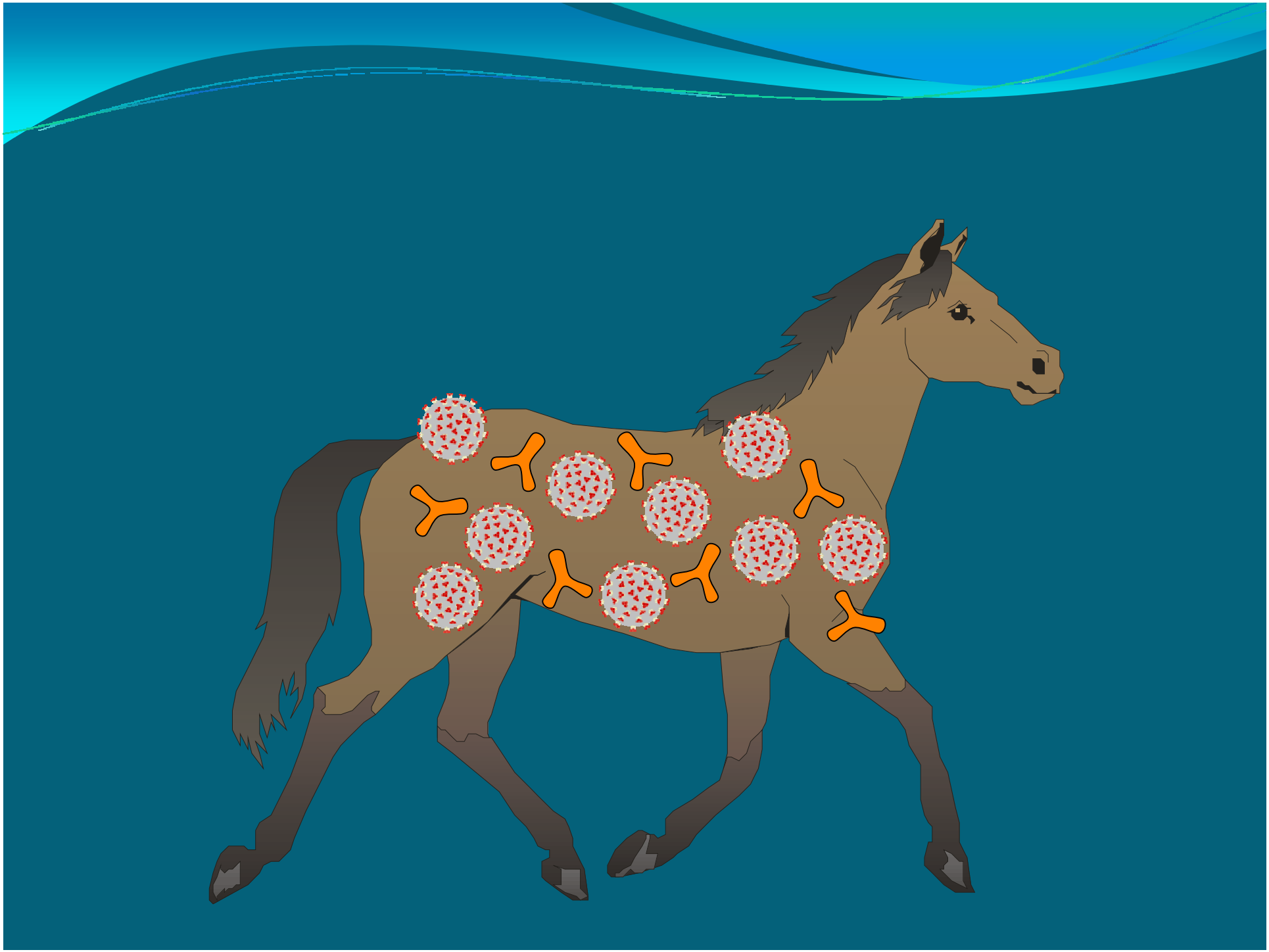
R. Frank Cook
Gluck Equine Research Center
University of Kentucky

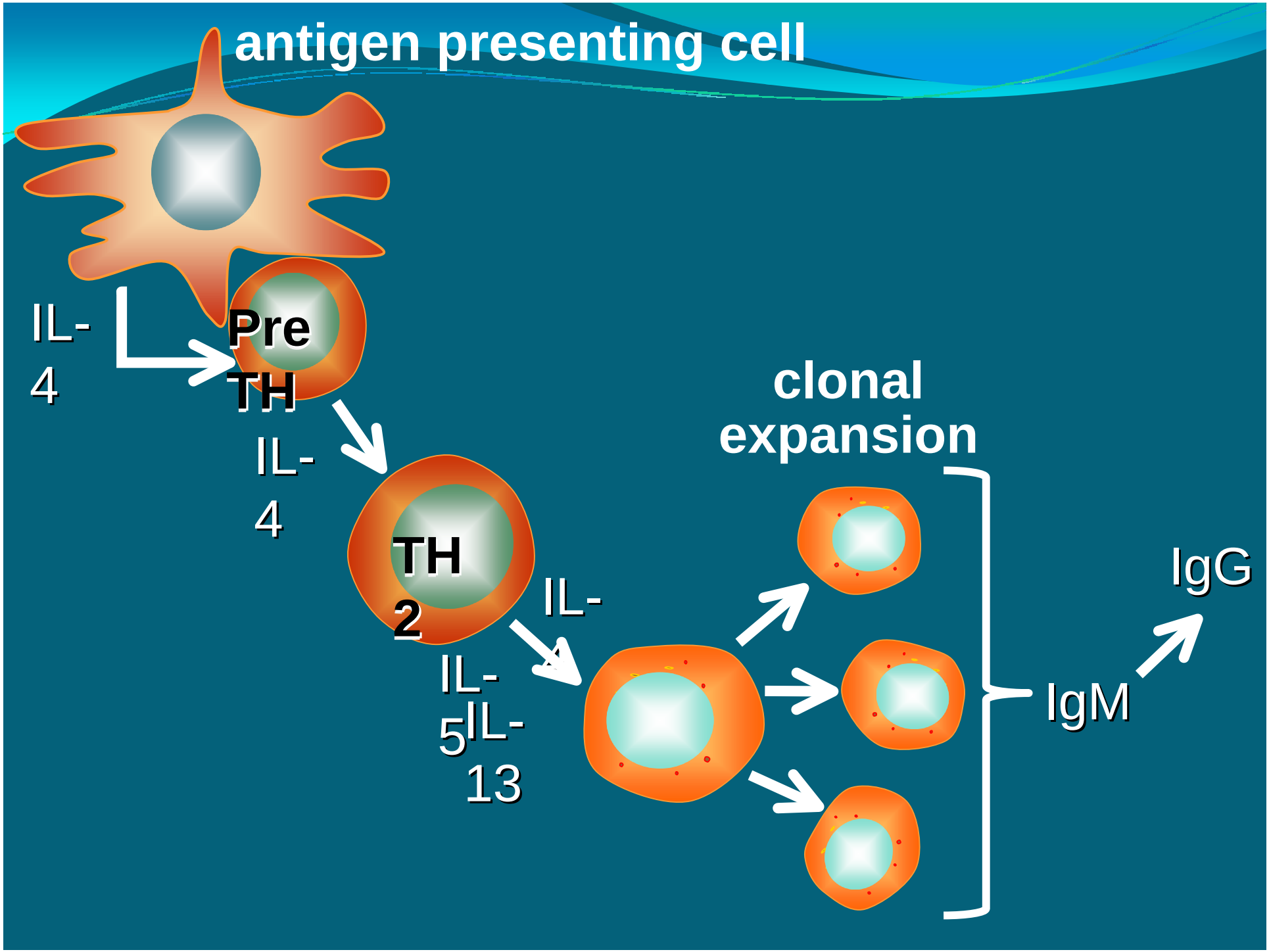
Why is There a Need for Molecular Detection of EIAV?

IAV: Serological Diagnosis



Indirect Method of Detection




antigen presenting cell
IL-4
Pre TH
IL-4
TH 2
IL-5
IL-13
clonal expansion
IgM
IgG

EIA

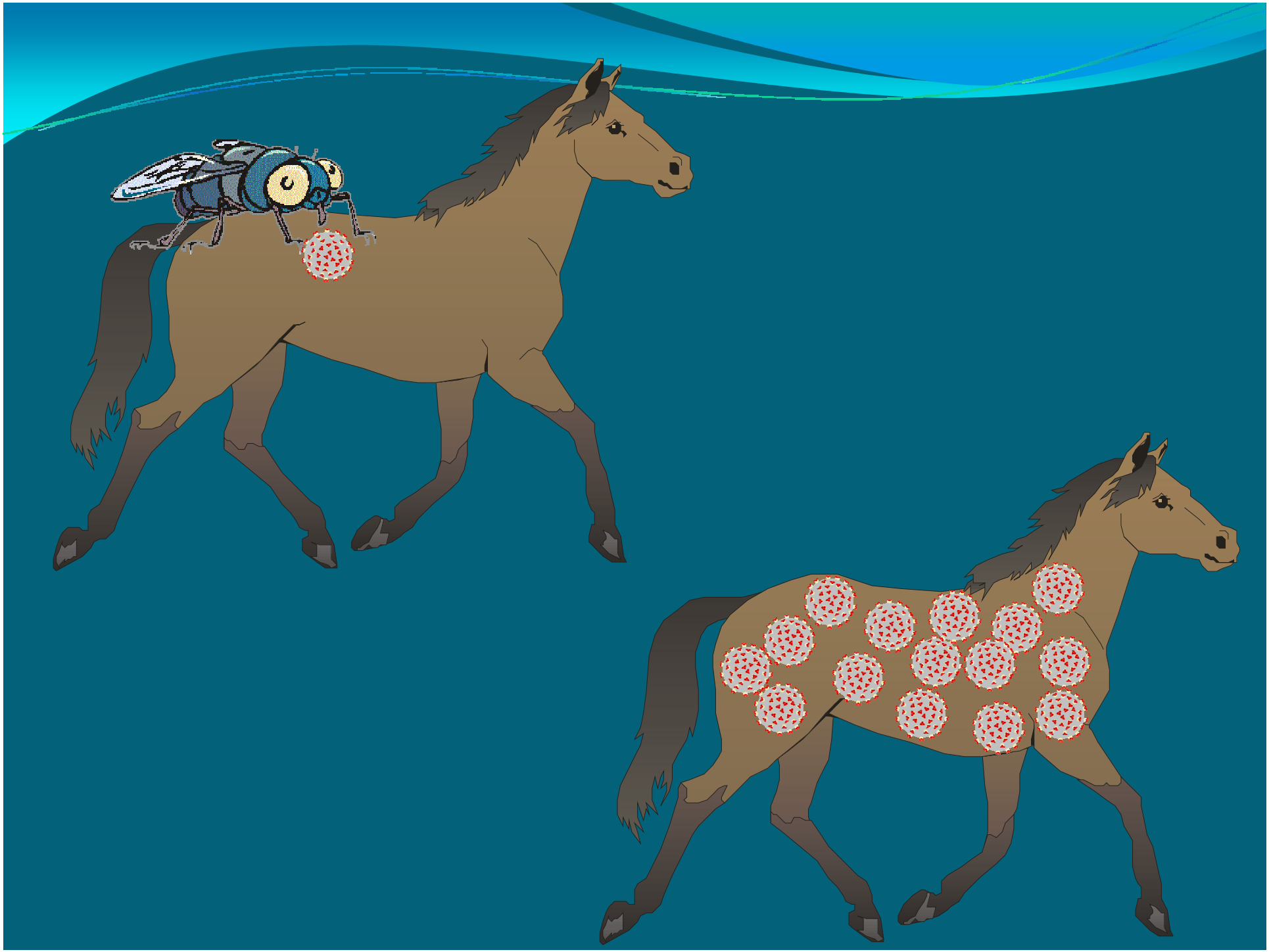
- Indirect Detection Disadvantages:
  - Incubation Period (AGID)
    - Most cases within 45 days (Issel & Cook 1993)
    - Longest reported 157 days (Cullinane et. al. 2007)

  - 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:

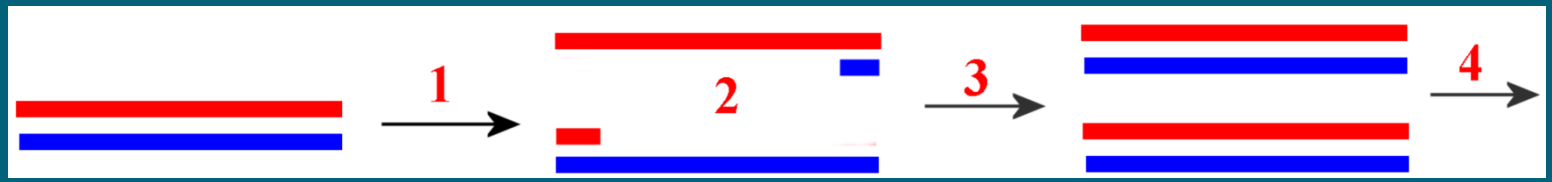
- Equine MØ
- Insensitive
- Highly Variable

Protein: Sensitivity

Nucleic Acid: vRNA/Proviral DNA

- PCR
- Isothermal

PCR



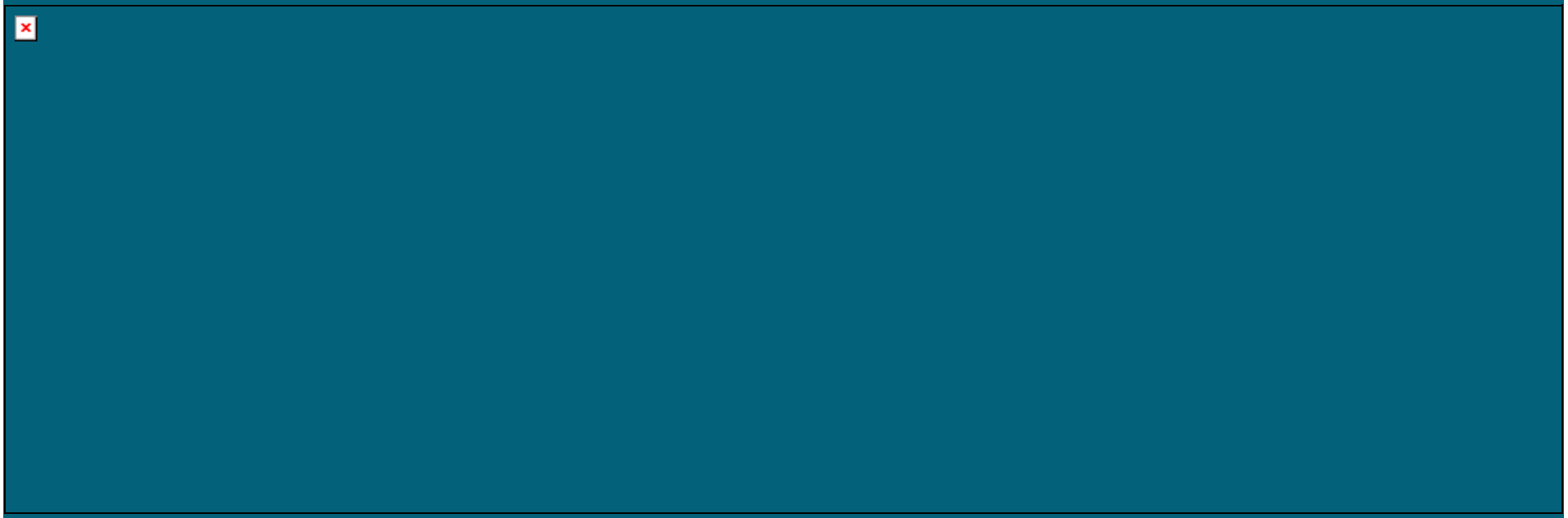
1: Denaturation (95°C)
2: Anneal (45-60°C)
3: Elongation (65-75°C)
4: “Repeat 1-3”

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



PCR Primer Mismatches

Pol

Primer TAGGAATTCTCGCC TTAACG

Virus ATCCTTAAGAGCGGAATTGC

Primer TAGGAATTCTCGCC

Virus A CCTTAA AGCGGAATTGC

A T

annealing temp

A/T -2°C G/C -4°C = -6°C

GCC

Primer TAGGAATTCTC

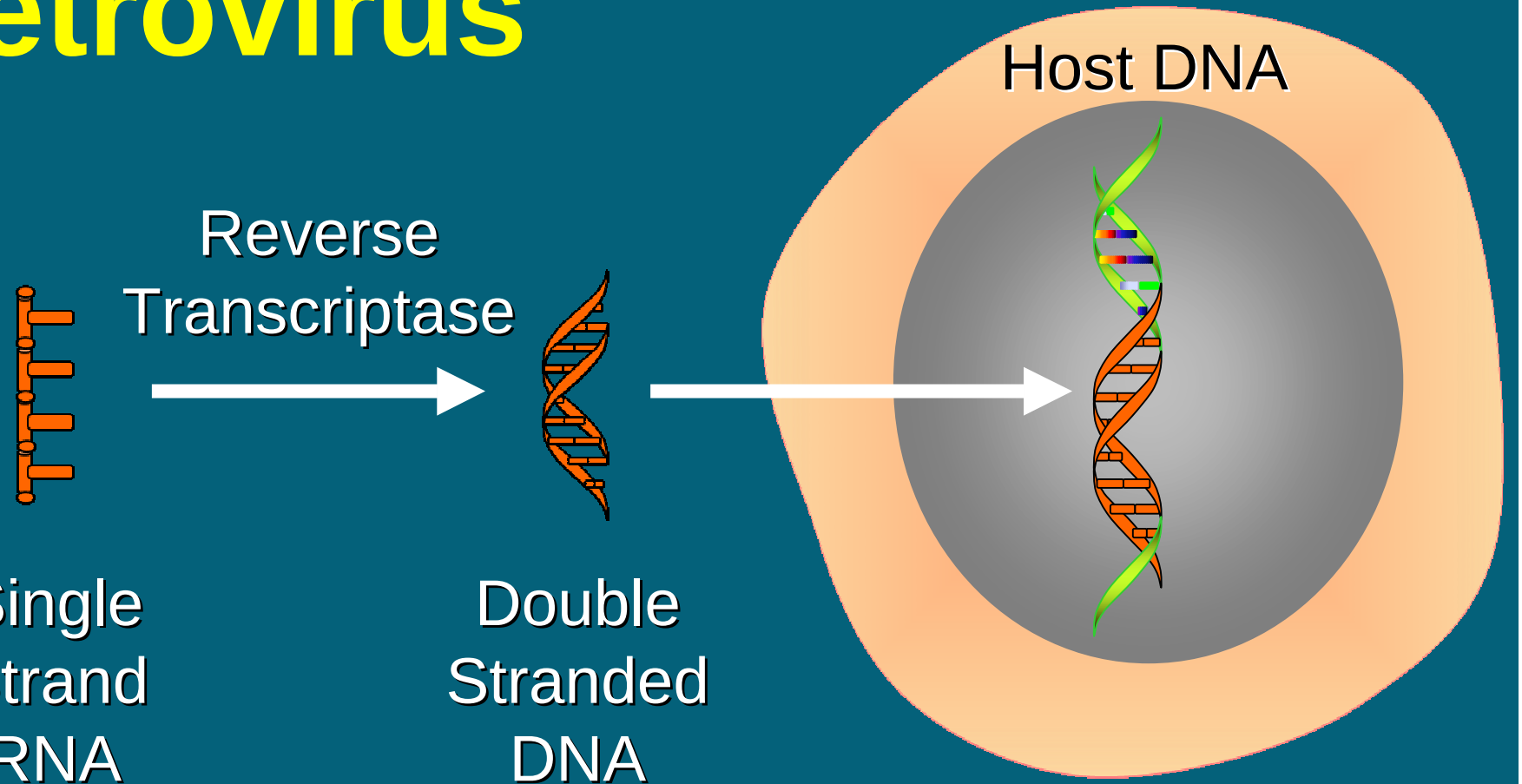
Virus ATCCTTAAGAGAAAAATTGC

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


Host DNA
Reverse
Transcriptase
Single
Strand
RNA
Double
Stranded
DNA
Viral
Genome
Provirus

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

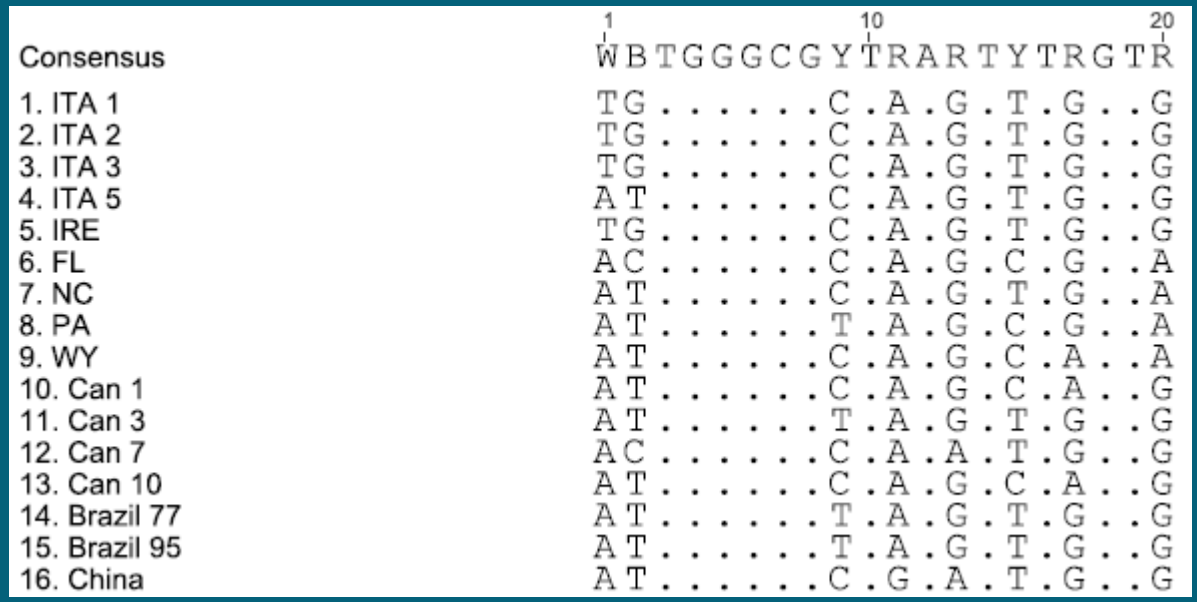
- Nagarajan and Simard, 2001
- Cappelli et. al., 2011
- Dong et. al., 2012
- Modified Cappelli

Samples: Argentina
26 AGID –ve
25 AGID +ve
3 Febrile

Template: 1µg PBMC DNA

TaKaRa Ex Taq™

Primer Variation



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 C: Cappelli et.al.
D: Dong 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

Conclusions

- PCR Primers reactive against unknown field strains
- N&S – OIE too strain specific?
- Cappelli, Dong, Modified: HIGH specificity; VERY LOW reactivity against endogenous retroviral elements
- LOW sensitivity: Primer mismatches?
  Detection limits??

STILL WORK TO DO