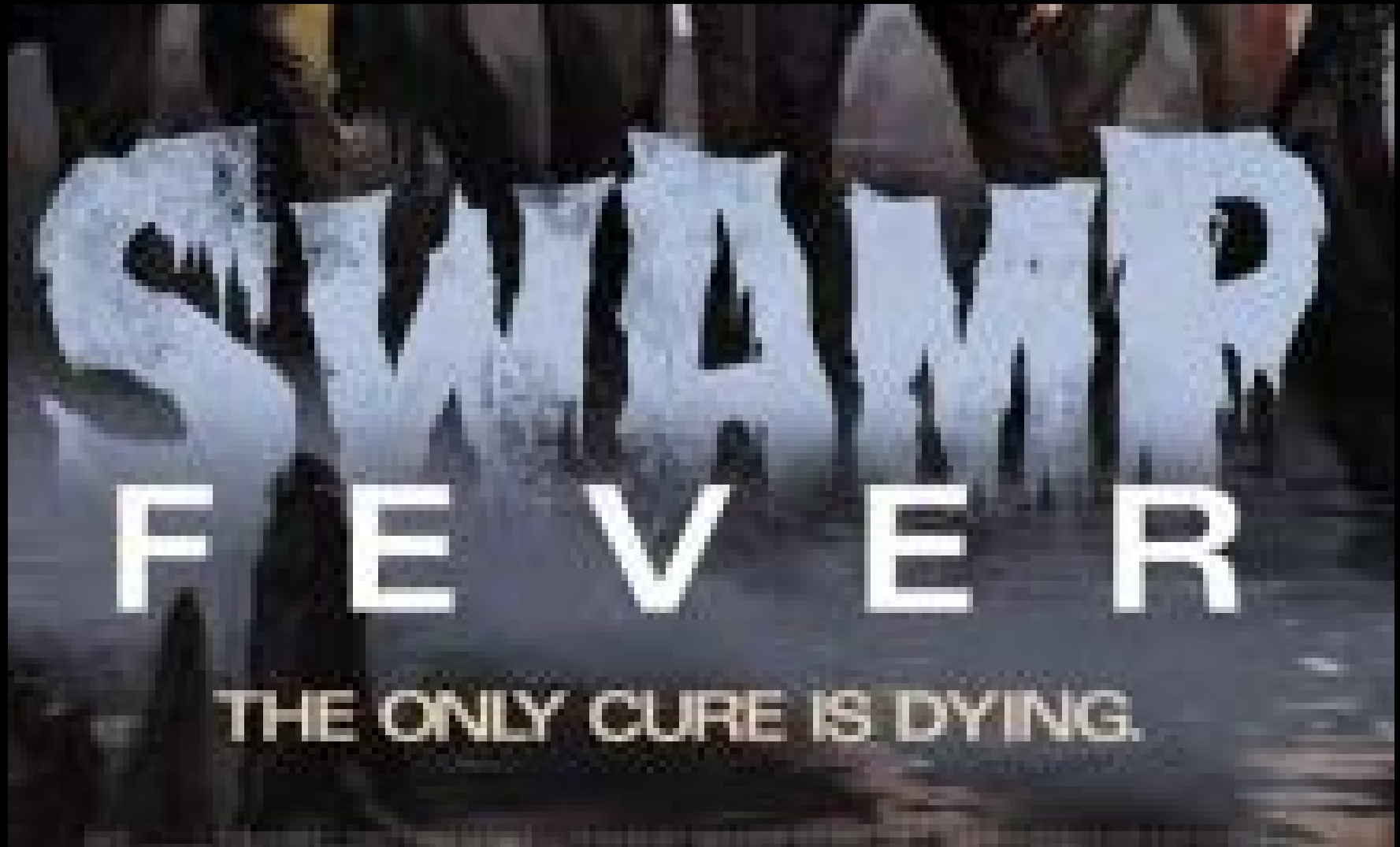



Equine Infectious Anemia

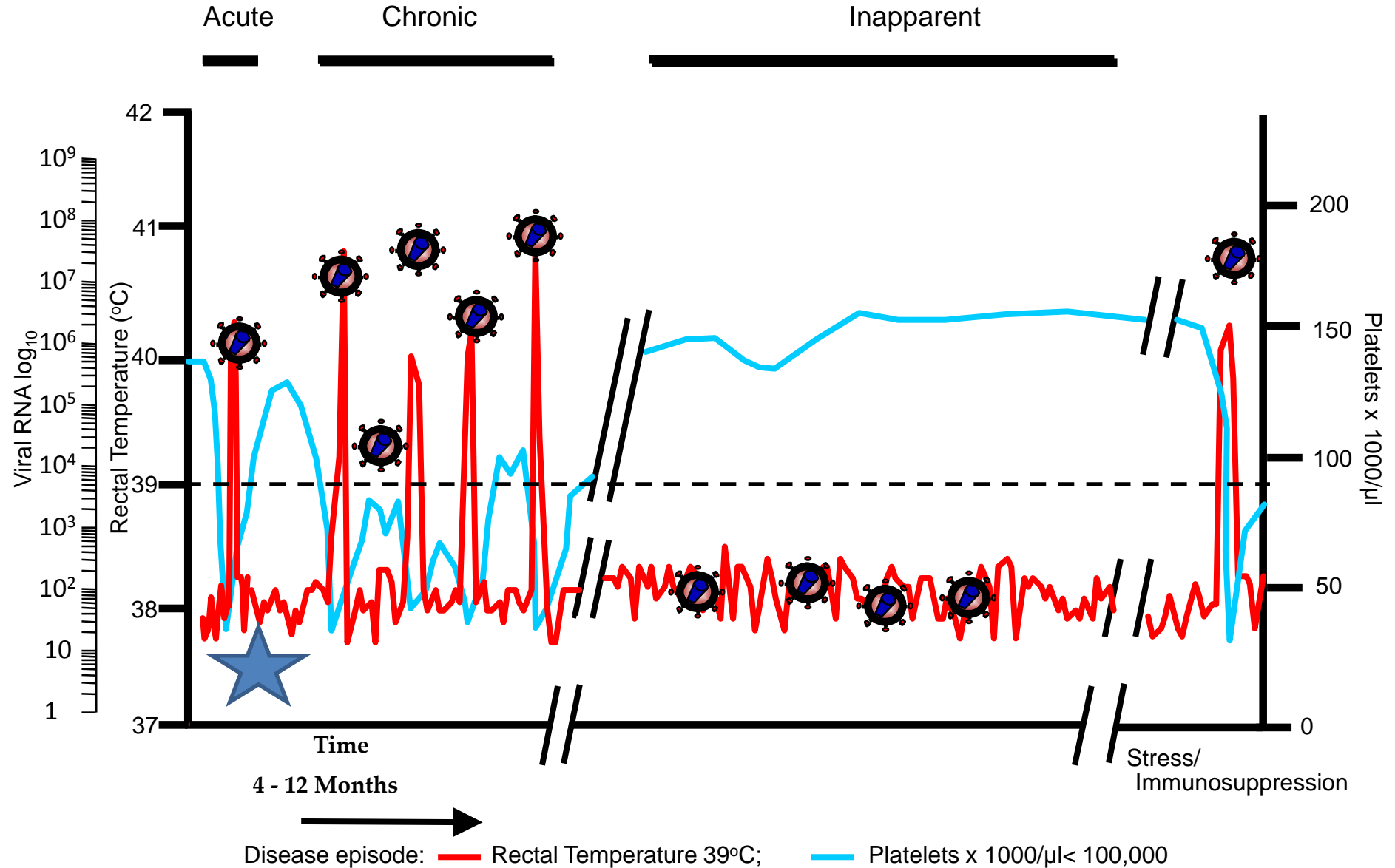


A photograph of the Gluck Equine Research Center building at the University of Kentucky. The building is a large, modern, light-colored structure with a series of vertical columns and windows. The name "THE DANWELL H. GLUCK EQUINE RESEARCH CENTER" is inscribed on the upper part of the building. In the foreground, there is a green lawn, a pond with a fountain, and some trees with autumn-colored leaves. The sky is clear and blue.

CJ Issel, DVM, PhD
University of Kentucky
Gluck Equine Research Center



Typical Clinical Course of EIAV Infections



EIA in the United States

1970: No diagnostic test

1972-79: >50,000 cases found

1980-2000: Numbers decrease

From 4,000/yr to less than 1,000

2013: Overtested mobile population

38 cases >\$1,500,000 each!

Untested reservoir

Diagnosis of EIA

Clinical signs	Not reliable	
Virus isolation	Not practical/possible	
Antibody detection	AGID	1970
	ELISA	1984
	Immunoblot	1984
Viral RNA/DNA	Gaining acceptance/use	

Gold Standard: Antibody detection in AGID
(correlation Horse Inoc-250ml)

Serologic Testing – Three Tier System

	Result	Report
ELISA first testing	NEG	NEG
	POS	
Test by AGID	POS	POS
	NEG	
Immunoblot	POS	POS
	NEG	NEG

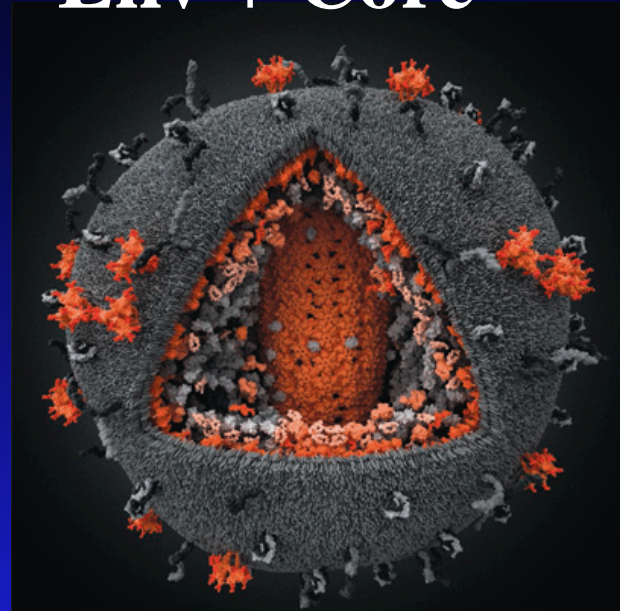
```
graph TD; ELISA[ELISA first testing] -- POS --> AGID[Test by AGID]; AGID -- POS --> Immunoblot[Immunoblot]; AGID -- NEG --> Immunoblot;
```

Serological Diagnosis of EIA

Antibody Tests for EIA

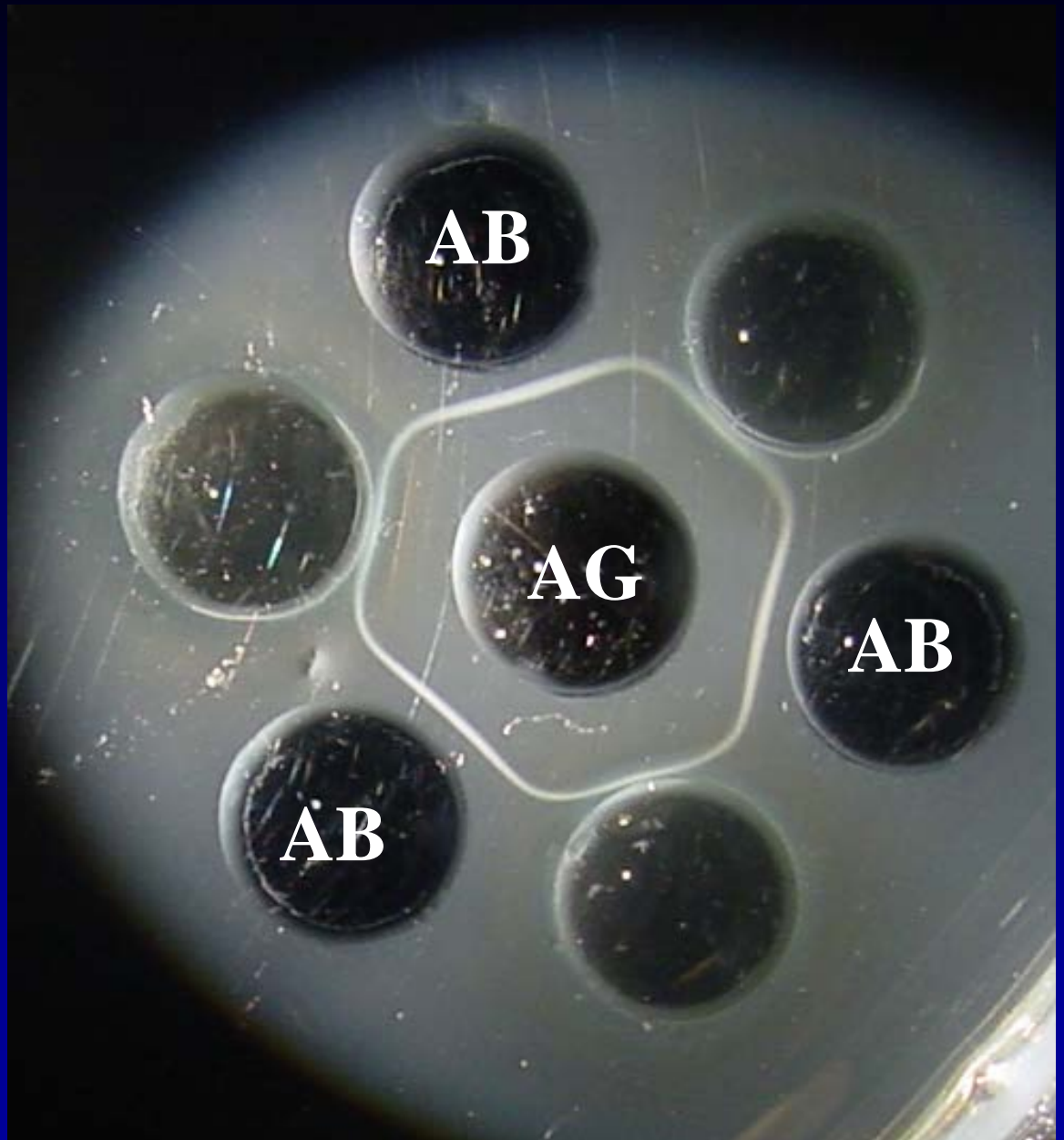
AGID (Coggins)	p26	Major core
4 ELISA kits (US)	p26	Core
Immunoblot	gp90, gp45, p26	Env + Core

Envelope more immunogenic
p26 >40% of virion: ~2000/
gp90-gp45: minor ~30/



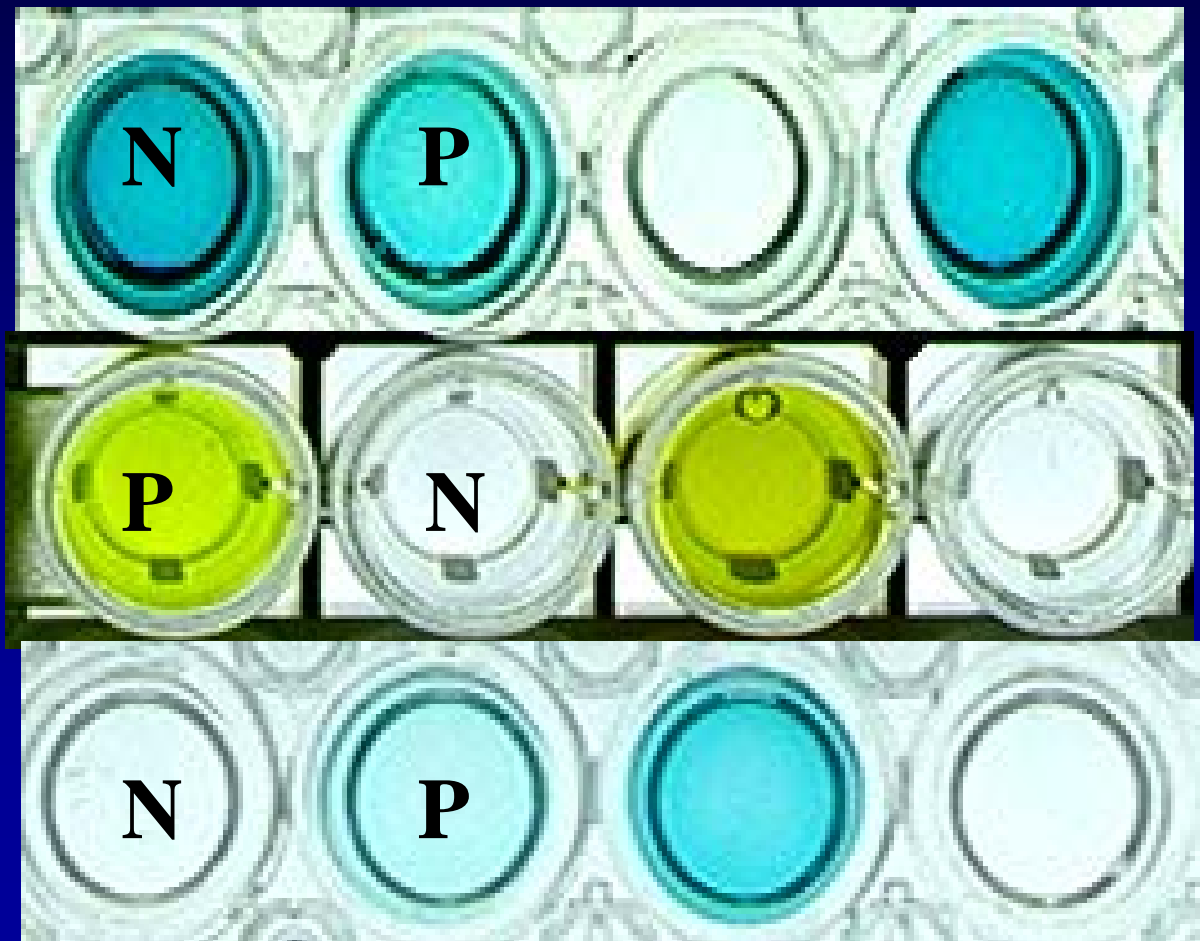
AGID
(Coggins)

POS



ELISA Test Reactions

Colors compared to Reference Controls
Spec reading makes it more objective



Immunoblot Testing for EIA

Virus grown, purified and SDS-heat treated

Separated into individual proteins

by relative molecular mass-PAGE

Transferred to membranes

Suspect serum tested at **1:20 dilution**

React with at least 2 major proteins?

Surface unit, transmembrane, major core

gp90

gp45

p26

Immunoblot Testing for EIA

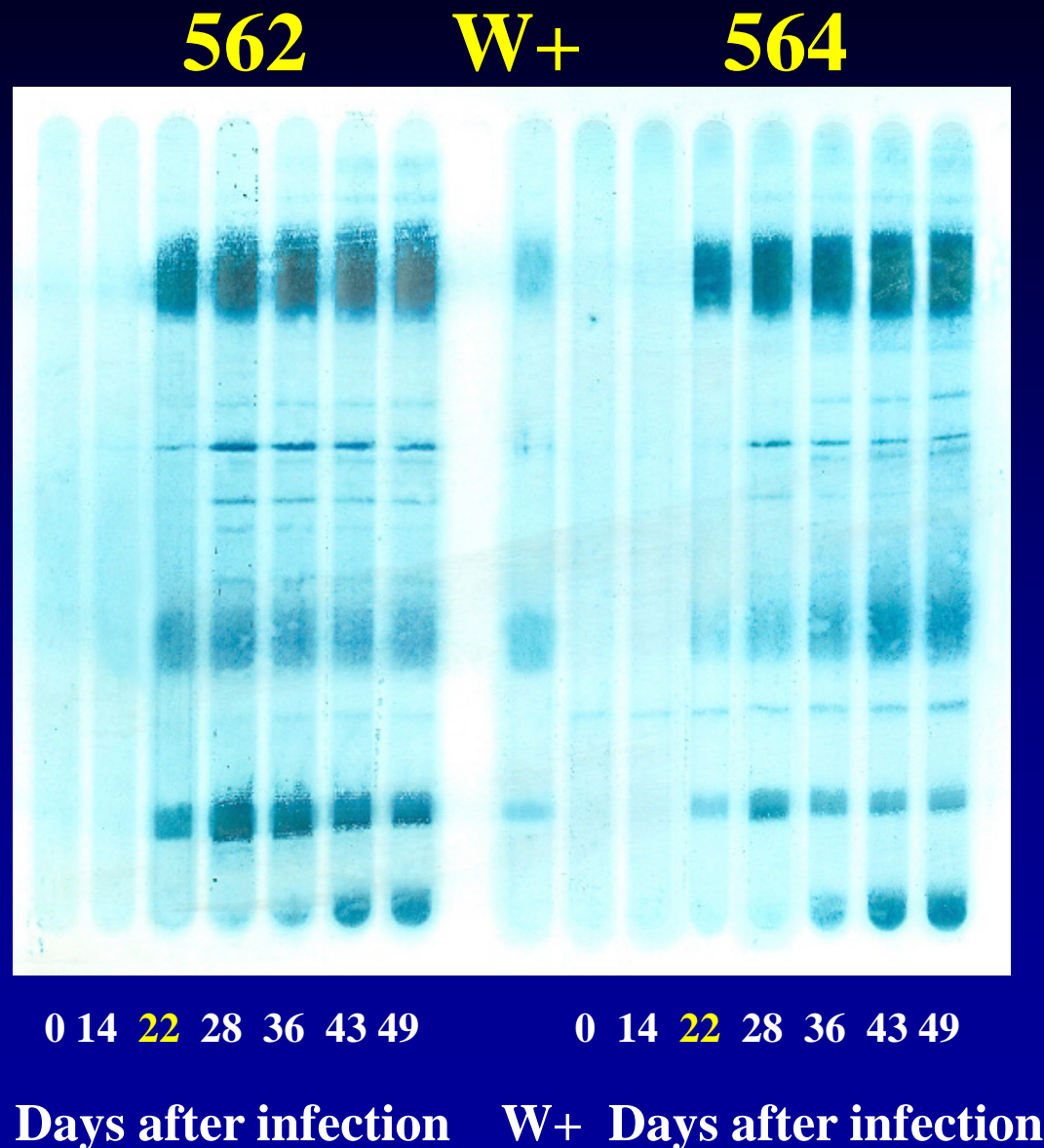
Responses of horses to
IV inoculation with
 10^3TCID_{50} of EIAV

gp90

gp45

p26

p15





Test

Expected Results

1

2

AGID

-

+

ELISA1

-

+

ELISA2

-

+

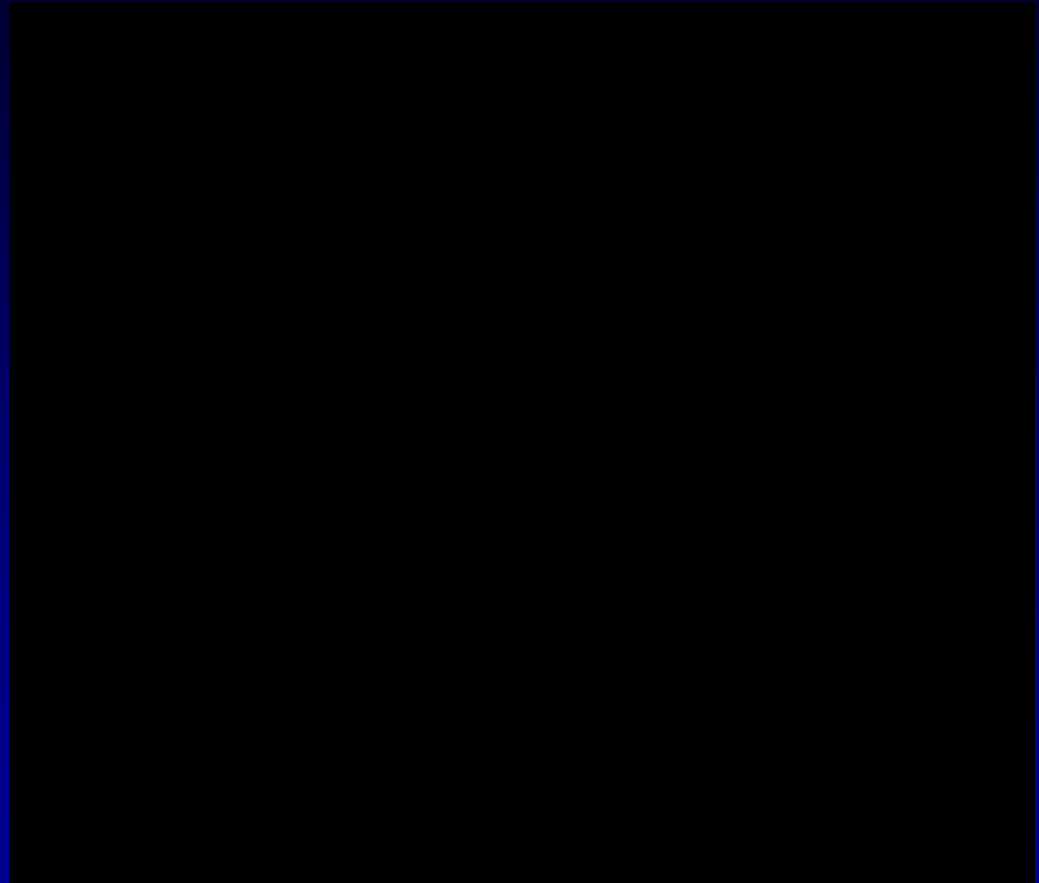
ELISA3

-

+

Immunoblot-

+



Rate

>99%

>99%

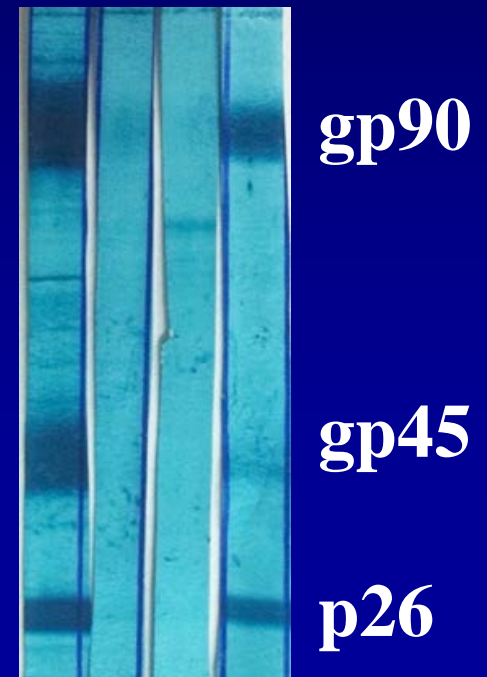
Serologic Testing for EIA

Usual Sample: total agreement in AGID/ELISA

Some: Interpreted AGID NEG / ELISA POS

Some: AGID misinterpreted and blot +

1 - - W+





Challenges in Serologic Diagnosis of EIAV Infections

180-210 days after infection – vaccine strain

AGID/ELISA/Blot











75% of vaccinates Positive in all 3 serologic assays

First antibody by 28-42 days

25% Negative by AGID: +/- in ELISA: + Blot

Challenges in Serologic Diagnosis of EIAV Infections

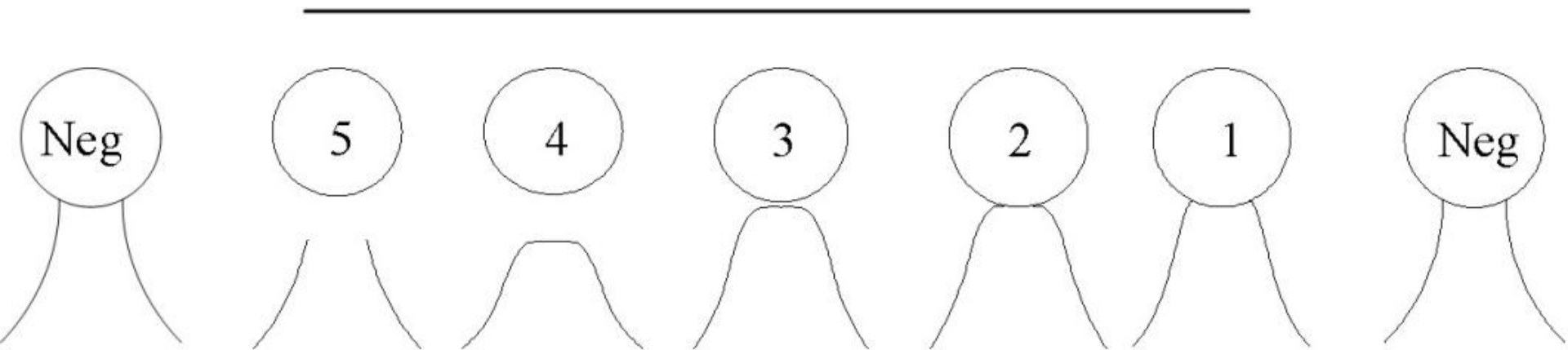
180-210 days after infection – vaccine strain

Animal	Virus ²	AGID	ELISAs Tests		Immunoblot		
			US kits ³	IT ⁴	p26	gp45	gp90
Experimental infections⁵			1/2/3				
C9	Yes	NEG	+/+/+	1:24			
C15	Yes	NEG	+/+/-	<1:6			
C16	Yes	NEG	+/+/-	<1:6			
C22	Yes	NEG	+/+/-	<1:6			
C23	Yes	NEG	+/+/+	<1:6			
B62	Yes	NEG	-/+/-	<1:6			
BT210	Yes	NEG	+/+/+	1:12			
C50	Yes	NEG	-/+/-	<1:6			
H46	Yes	NEG	+/+/-	<1:6			
H32	Yes	NEG	+/+/-	1:6			



AGID (Coggins) Test Reactions Expected

Positive Reactions




<1 ≥ 10 7 4 2 1 <1

**Amount of Antibody to p26
With constant antigen**



Serologic Responses to EIAV – Reference W+

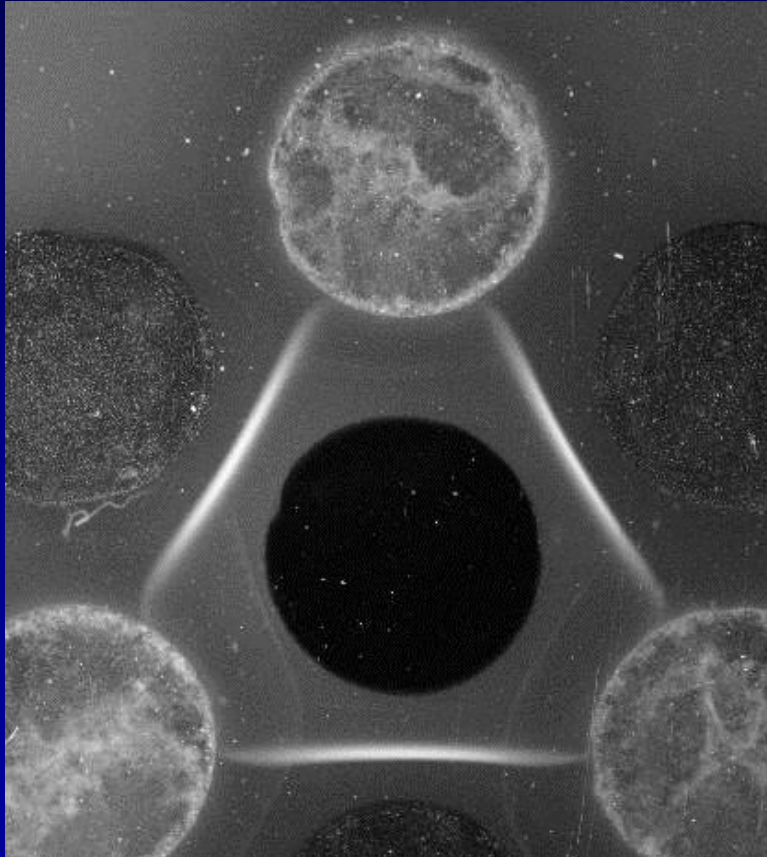
Animal	Virus ²	AGID	ELISAs Tests		Immunoblot		
			US kits ³	IT ⁴	p26	gp45	gp90
Reference Positive Serums							
Flicker W+	Yes	NEG	+/+/+	1:8			
USDA W+	???	1	+/+/+	1:48			



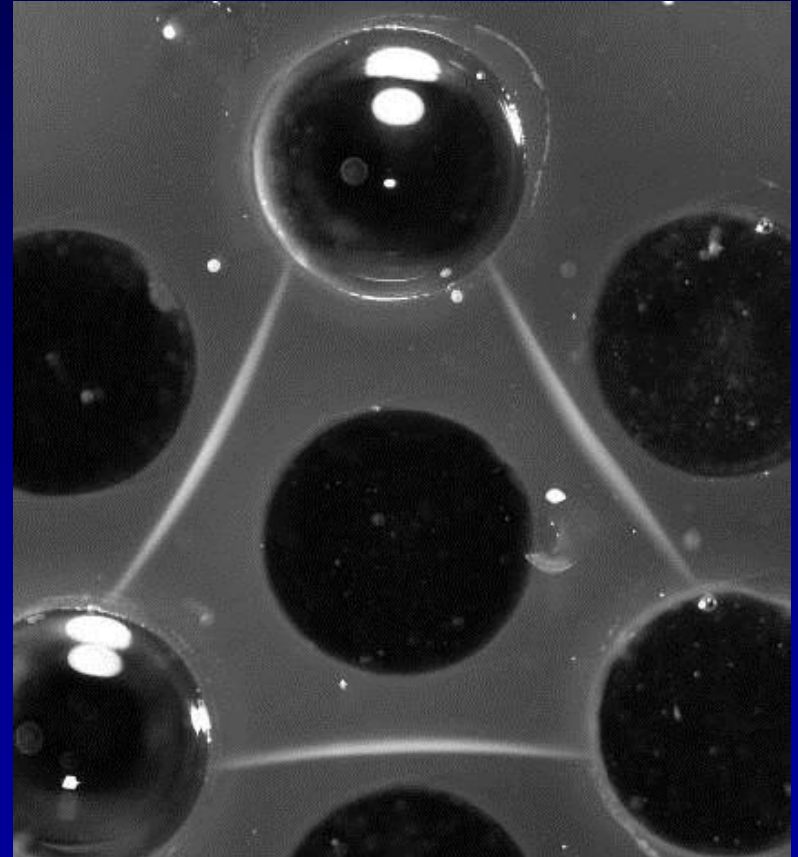
“Weak Positive” AGID

Accurate Interpretation Required

Ref W+ USDA



UK W+Flicker



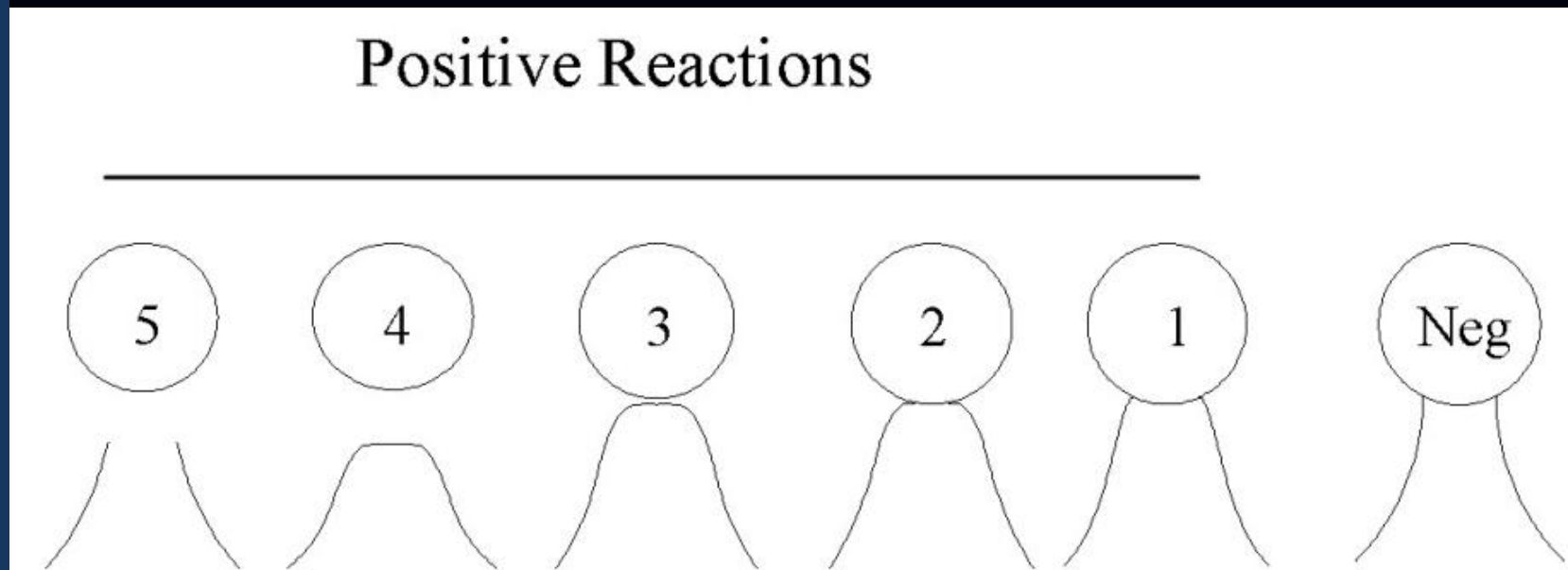
AGID Test Parameters Compared How They Impact Accuracy

	1970's	Today
Antigen source	Virus	Recombinant
Template used	Large	Smaller
Antigen costs	Higher	Low
Ease in reading	Low	Higher

**Expectations: Higher rate of False-NEG
AGID reports with rec-antigen kits**



AGID (Coggins) Test Reactions Expected



<< < 1 > >> >>>

**Relative Amount of p26 Antigen
With constant test serum**



Serological Diagnosis of EIA

Basic research defines the need for improvement

Applied studies prove value of model:

3 tier strategy

**A cooperation between researchers at the
University of Kentucky and the staff of the
National Reference Centre for Equine Infectious
Anemia (IZS-Lazio e Toscana) during
surveillance for EIA: 2007-2010**

Serology Italy – 3 Tier Lab Testing

	Number	%
Samples	96,468	
+ ELISA	331	0.36
+ E & AGID	124	0.13
Discrepant Samples	207	0.21
+ Immunoblot	25	
- Immunoblot	182	
False + ELISA	182/96,468	0.19
False – AGID	25/96,468	0.026

Vet Record (2013)



Three Tier Strategy: Field Testing Italy

First tier

ELISA

Second

E+ AGID

Third

E+A+Blot

Is there a need to adopt it more widely?

**Yes, 17% (25/149) of equids AB+ for EIA
missed by routine AGID in this survey**



Three Tier Strategy: Field Testing

Comments-Perspective:

- 1 -Official recognition of limitations of AGID
- 2 -Field proficiency: routine performance
- 3 -Use investment by the industry wisely
In US, >US\$70,000,000/yr

EIA Control: 2010 Indicated changes

**Test by risk,
not regulation**

**New lab paradigm:
3 tier system**



