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

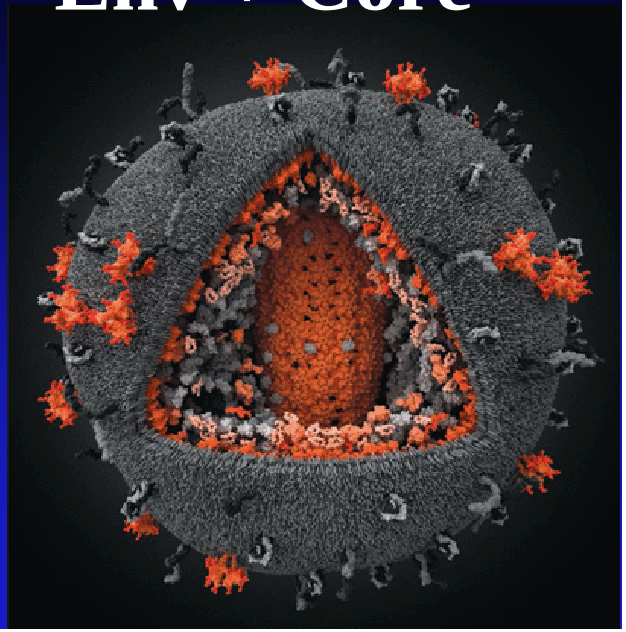
Serologic Testing – Three Tier Lab System

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

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/



Immunoblot Testing for EIA

Virus grown, purified and SDS-heat ttmt
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

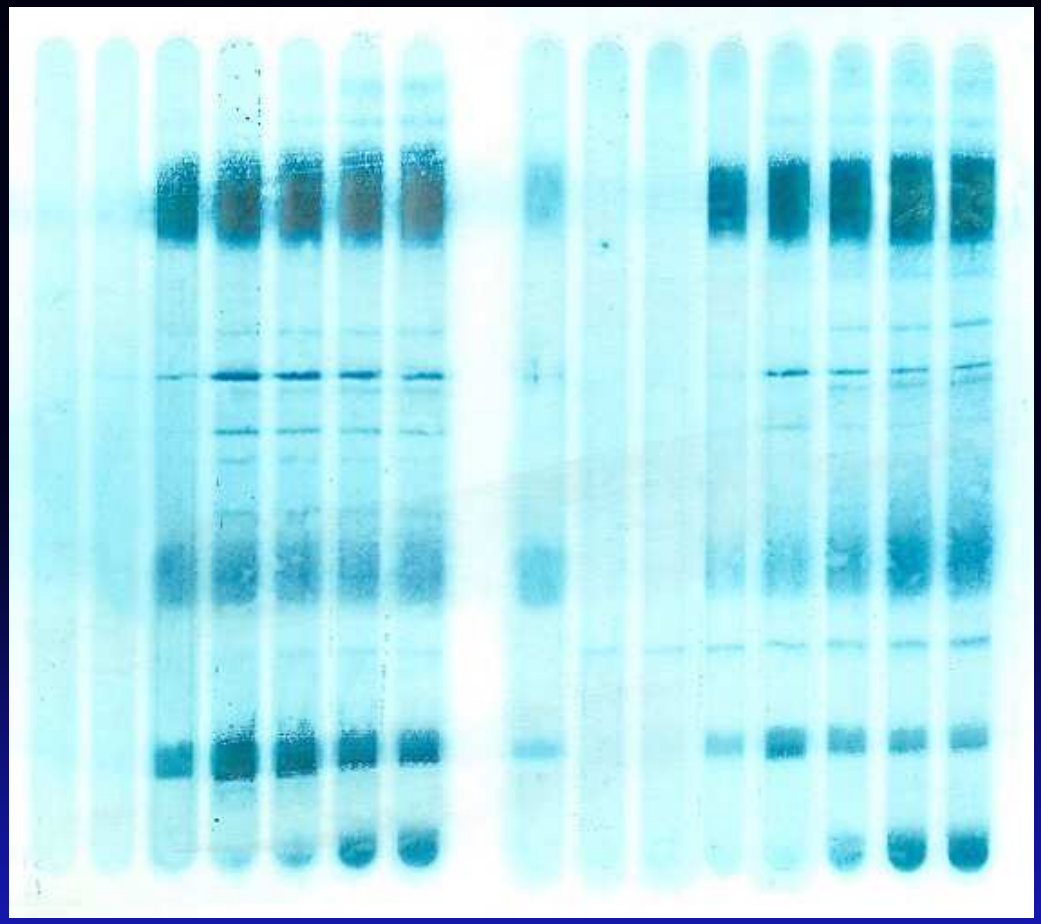
Responses of horses to IV inoculation with $10^3 TCID_{50}$ of EIAV

gp90

gp45

p26

p15



Test	Expected Results	1999
	1	7
AGID	-	+
CELISA	-	+
Vira-CHEK	-	+
SA-ELISA	-	+
Immunoblot	-	+
Rate	>99%	>99%

Reservoirs/Risks/Need: 1999

Test all equids: only ~30% tested

Estimated error rate:

- False-negative reactors: ~1% of POS
- Estimated lab errors: ~1%

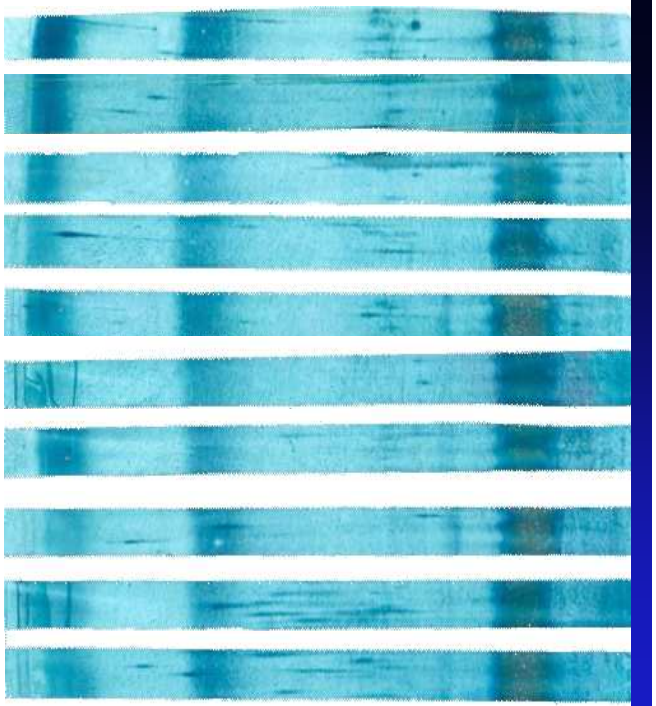
Estimated overall error rate: ~2% of true +

Miniscule compared to untested reservoir

What have we learned since then?

Challenges in Serologic Diagnosis of EIAV Infections 180-210 days after infection – vaccine strain

Animal	Virus[2]	AGID	ELISAs Tests		Immunoblot		
			USkits[3]	IT[4]	p26	gp45	gp90
Experimental infections[5]			**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			



Major issues: 1999

Subjective AGID results:

- **Same as in 1974!**
- **Personnel turnover?**
- **Eyesight fails?**
- **Intense light source?**
- **Others?**

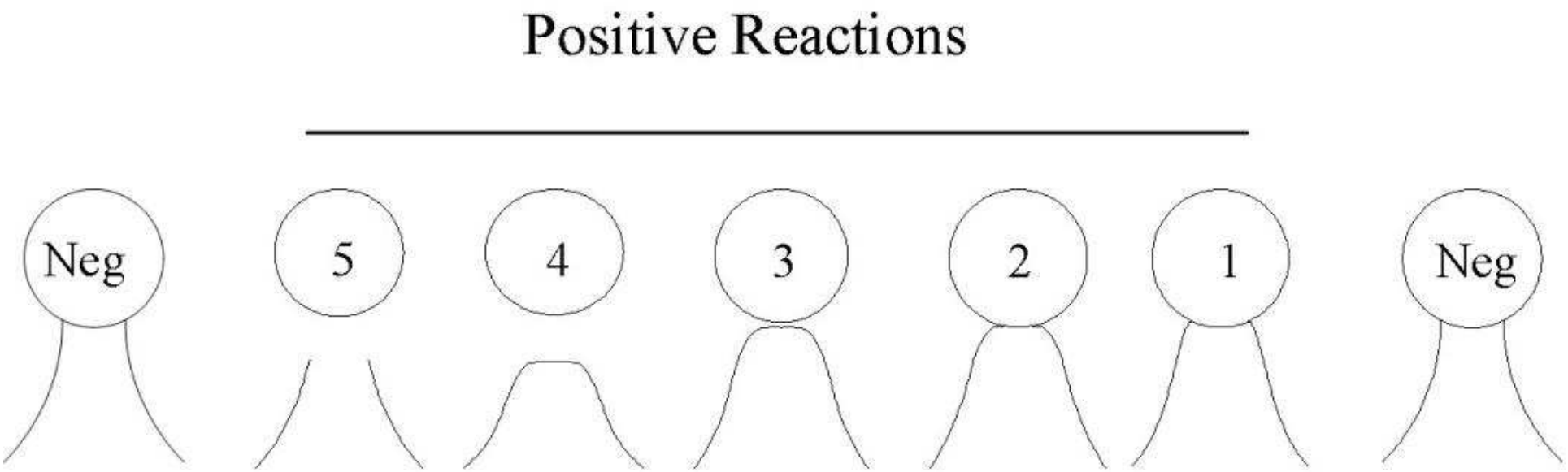
What has changed?

AGID Test Parameters Compared
How They Impact Accuracy

	1970's	Today
Antigen source	Virus	Recombinant
Template used	Large	Smaller
(Brazil		Slides – micro)

Expectations: Higher rate of False-NEG AGID reports with rec-antigen kits and smaller format.

AGID (Coggins) Test Reactions Expected

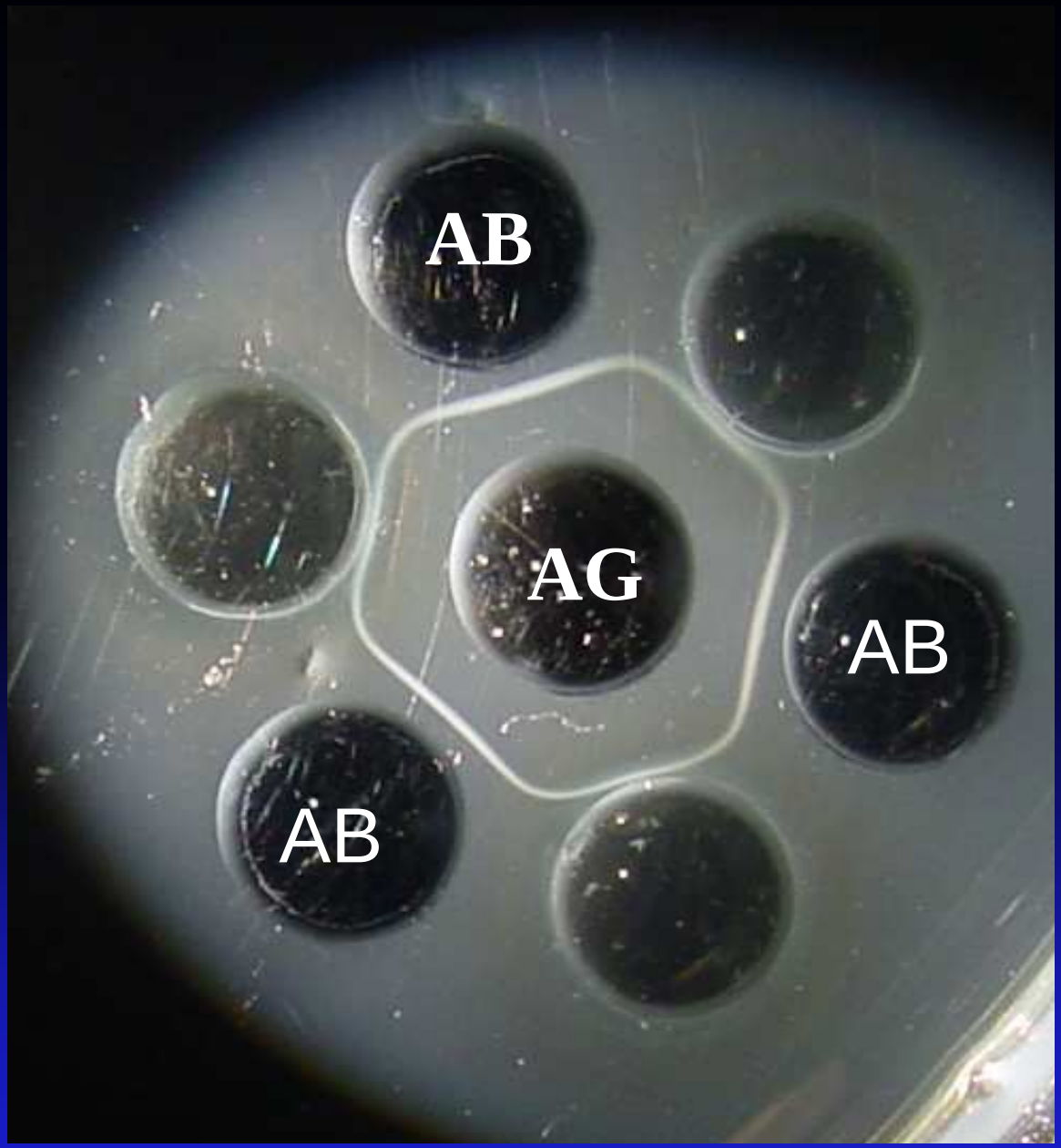


<1 ≥ 10 7 4 2 1 <1

Amount of Antibody to p26
With constant antigen

AGID (Coggins)

POS



“Weak Positive” AGID
Accurate Interpretation Required

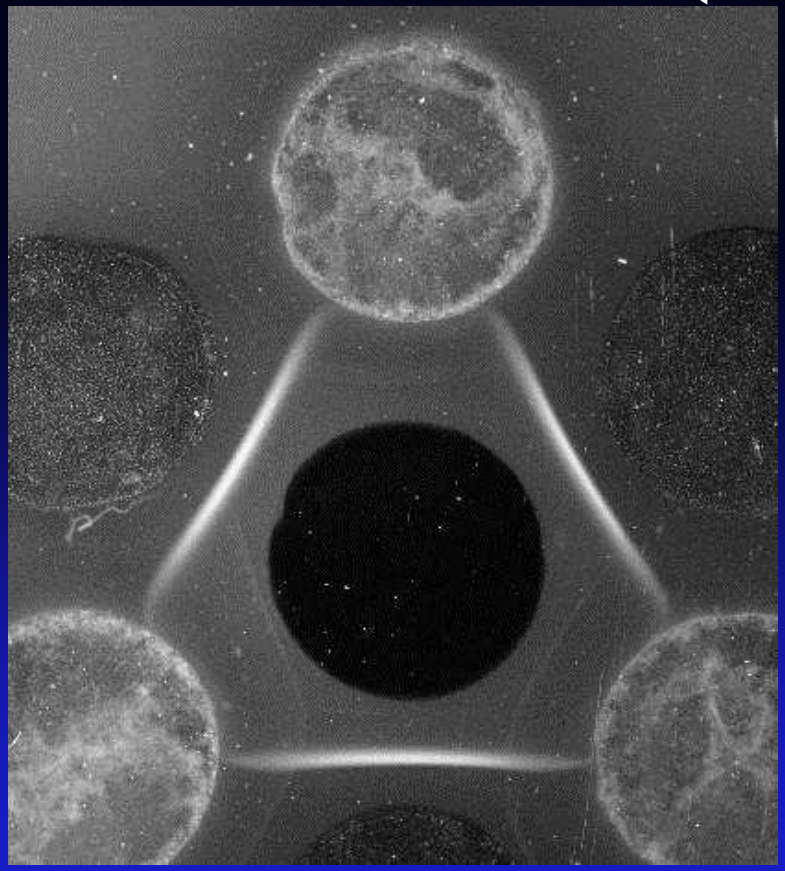
Ref W+
USDA
(older)

Field
Sample

Read at
48 hours

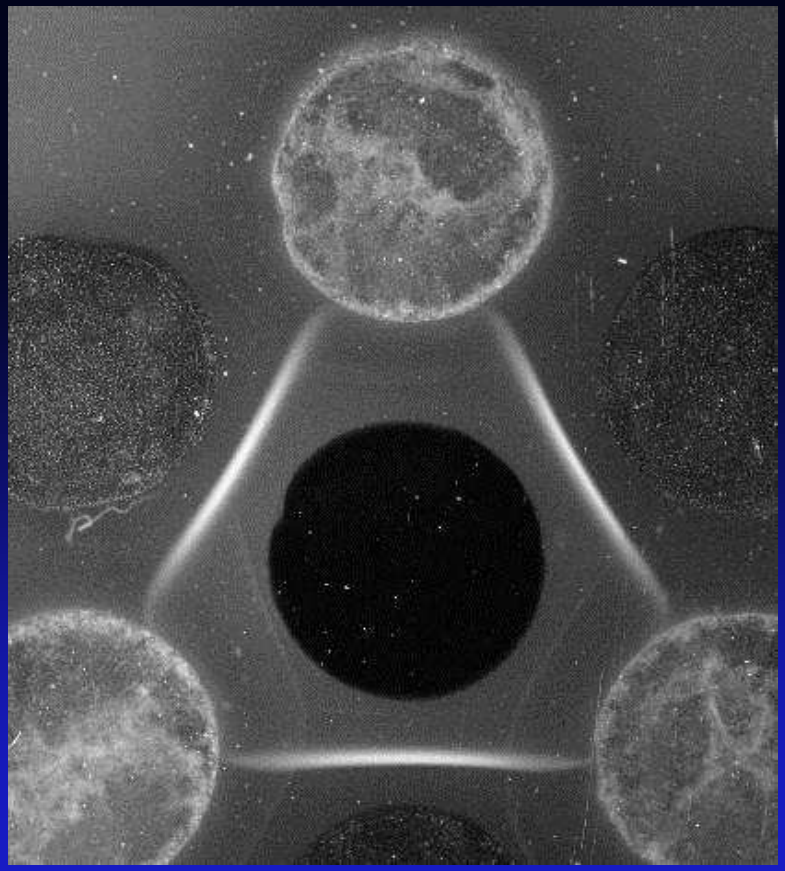
“Weak Positive” AGID
Accurate Interpretation Required

Ref W+ USDA (newer)

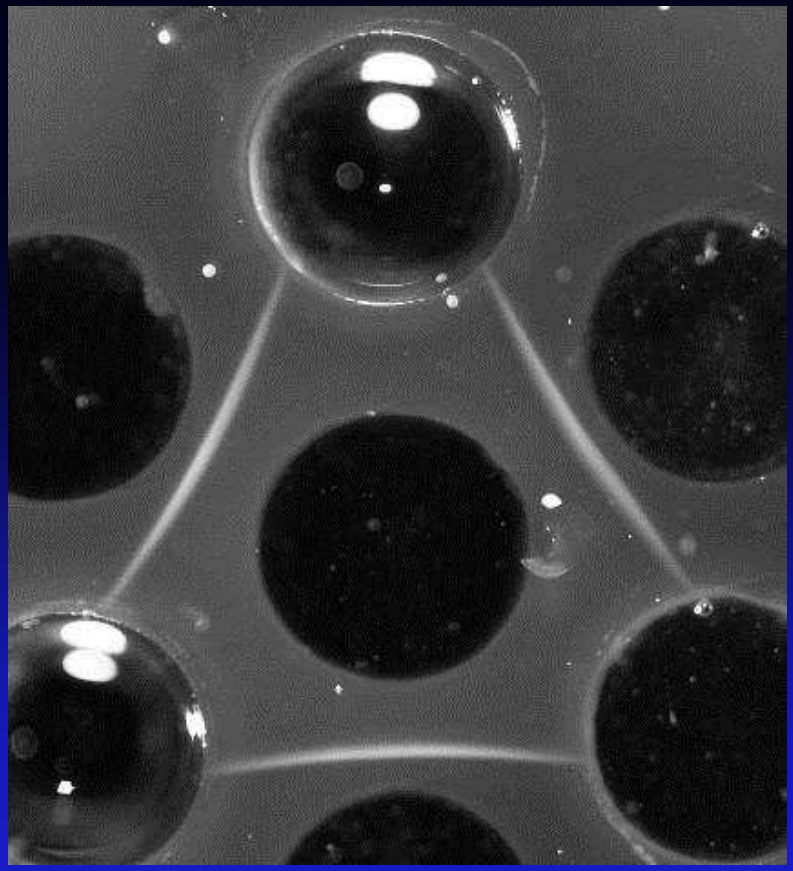


“Weak Positive” AGID
Accurate Interpretation Required

Ref W+ USDA



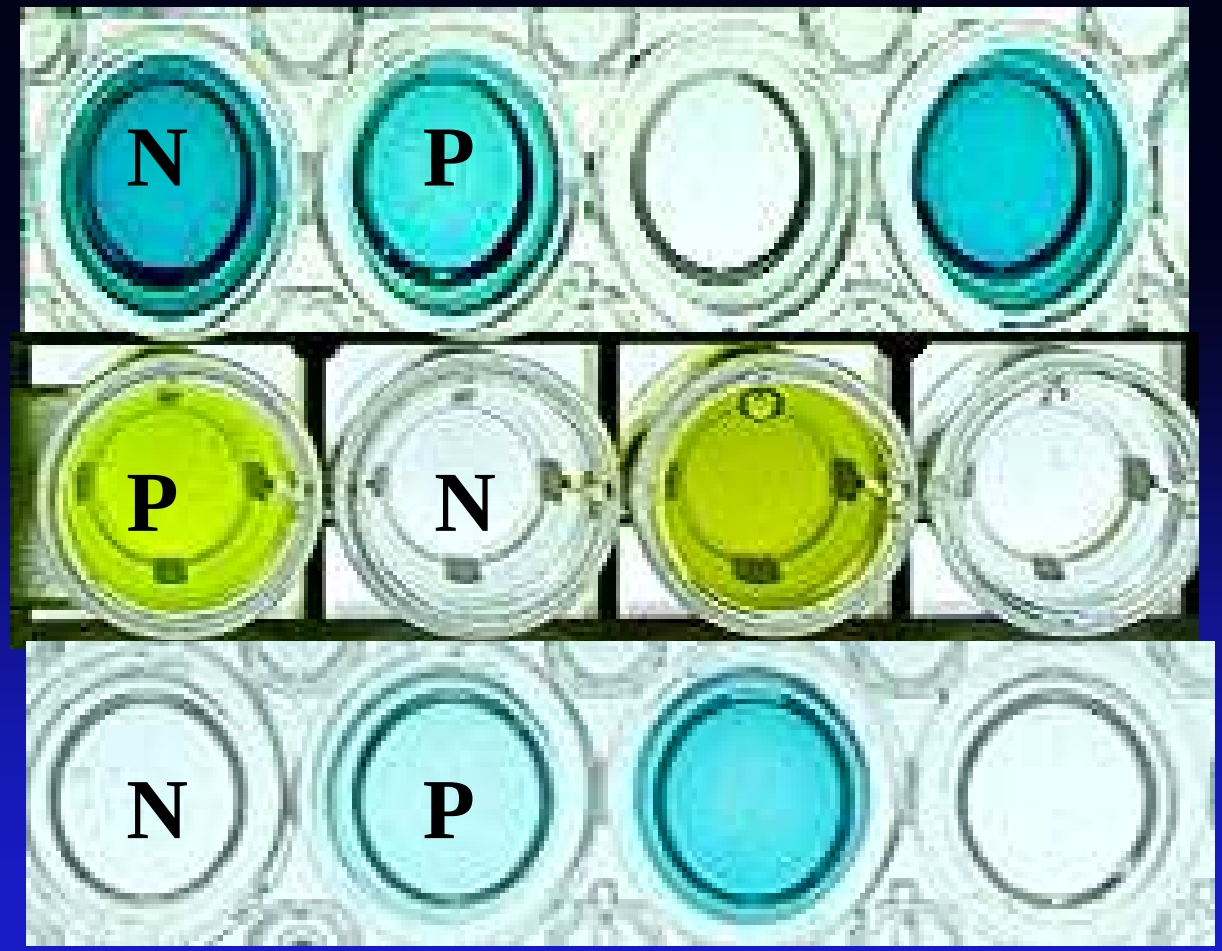
UK W+Flicker



ELISA Test Reactions

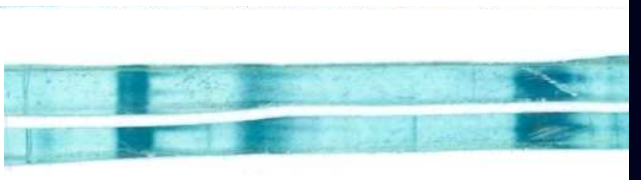
Colors compared to Reference Controls

Spec reading makes it more objective



Serologic Responses to EIAV – Reference W+

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



Pilot Study for Three Tier Strategy USDA

First tier	Private labs	ELISA only
Second	Referral labs	E+ AGID
Third	Reference lab	E+A+Blot

Maybe up to 30% missed by old strategy

Three Tier Strategy adopted by Oklahoma

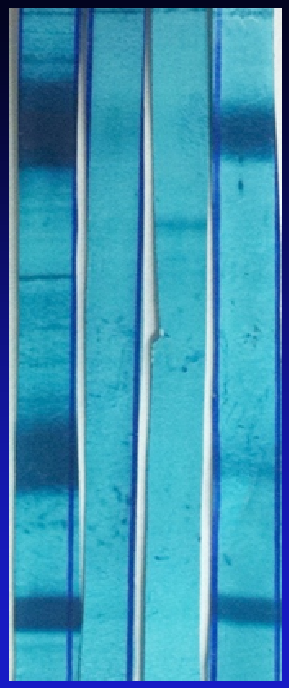
Serologic Testing for EIA

Usual Sample: total agreement in AGID/ELISA

Some: Interpreted AGID NEG / ELISA POS

Some: AGID misinterpreted and blot +

1 - - W+



Serologic Testing for EIA

Usual Sample: total agreement in AGID/ELISA

Some: Interpreted AGID NEG / ELISA POS

Rare: ELISA and Blot POS but AGID NEG

Because sample result misinterpreted

Or because antibody level too low

How many?

Three Tier Strategy: Field Testing Italy 2007-2010
All testing at one laboratory

First tier	ELISA
Second	E+ AGID
Third	E+A+Blot

Is there a need to adopt it more widely?

Serology Italy – 3 Tier Lab Testing

	Number	%
Samples	96,468	
+ ELISA	331	0.36
+ E & AGID	124	0.13

Serology Italy – 3 Tier Lab Testing

	Number	%
Samples	96,468	
+ ELISA	331	0.36
+ E & AGID	124	0.13
Discrepant Samples	207	0.21

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	12%	

Serology Italy – 3 Tier Lab Testing

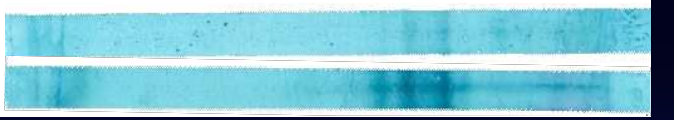
	Number	%
Samples	96,468	
+ ELISA	331	0.36
+ E & AGID	124	0.13
Discrepant Samples	207	0.21
+ Immunoblot	25	
- Immunoblot	182	

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

Serologic Responses to EIAV – Field Samples False+ ELISAs

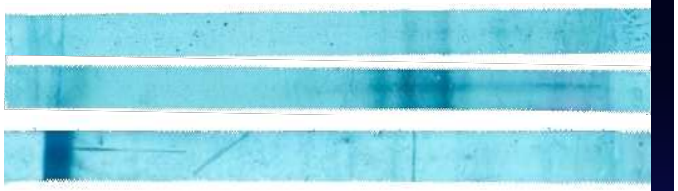
Animal	Virus[2]	AGID	ELISAs Tests		Immunoblot		
			USkits[3]	IT[4]	p26	gp45	gp90
Judged False Positive							
BG Filly 11/94	ND	?	-/-/+	<1:6			
BG filly 1/95	ND	NEG	-/-/+	<1:6			



React with <2 major proteins of EIAV

Serologic Responses to EIAV – Field Samples
False+ ELISAs

Animal	Virus[2]	AGID	ELISAs Tests		Immunoblot		
			USkits[3]	IT[4]	p26	gp45	gp90
Judged False Positive							
BG Filly 11/94	ND	?	-/-/+	<1:6			
BG filly 1/95	ND	NEG	-/-/+	<1:6			
Sugar	ND	NEG	+/+/+	1:96			



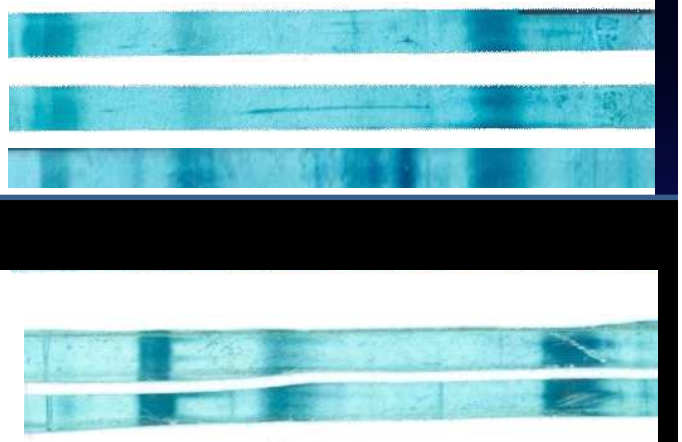
React with <2 major proteins of EIAV

Reactions such as Sugar are extremely rare!

Requires immunoblot for confirmation

Serologic Responses to EIAV – Field Samples

Animal	Virus[2]	AGID	ELISAs Tests		Immunoblot		
			USkits[3]	IT[4]	p26	gp45	gp90
Field samples							
Judged True Positive							
Jethro 7/02	ND	NEG	+/+/-	<1:6			
Jethro 3/11	ND	NEG	+/+/-	<1:6			
Shadow 8/02	ND	NEG	+/+/+[6]	<1:6			
Reference Positive Serums							
Flicker W+	Yes	NEG	+/+/+	1:8			
USDA W+	???	1	+/+/+	1:48			



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

Vet Record (in press)

Three Tier Strategy: Field Testing

Comments-Perspective:

1 -Official recognition of limitations of AGID

2 -Field proficiency test: on routine performance

3 -EU 1st Lab proficiency test results surprising

Better test of accuracy than USDA

"If too many do not pass it was a bad test"

4 -Use investment by the industry wisely

In US, >US$70,000,000/yr

EIA Control: 2012 Indicated changes

Test by risk, not regulation

New lab paradigm: 3 tier strategy