AGID (Coggins) Testing Considerations: Follow Protocols Exactly

Fill the wells properly.

All wells underfilled

Slightly concave meniscus

All wells filled properly*

No meniscus–absolutely flat

Top well overfilled

Convex meniscus–spills occur

*With 15 mL of agar in each level plate, each well should accept between 65 and 70 μL of sample.

Use optimal agar.

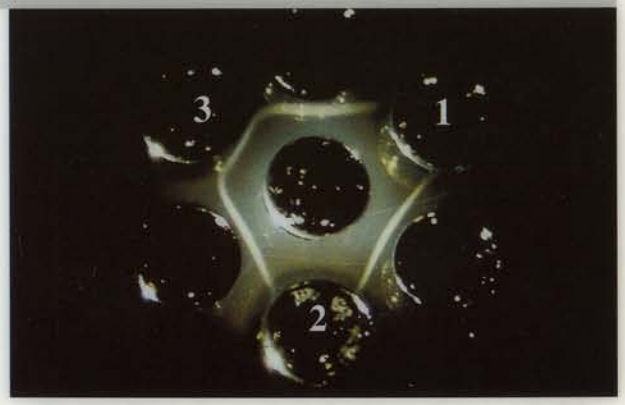
Optimal

Caramelized (slightly brown)

Use an intense light source.

Reduce ambient light for better viewing.

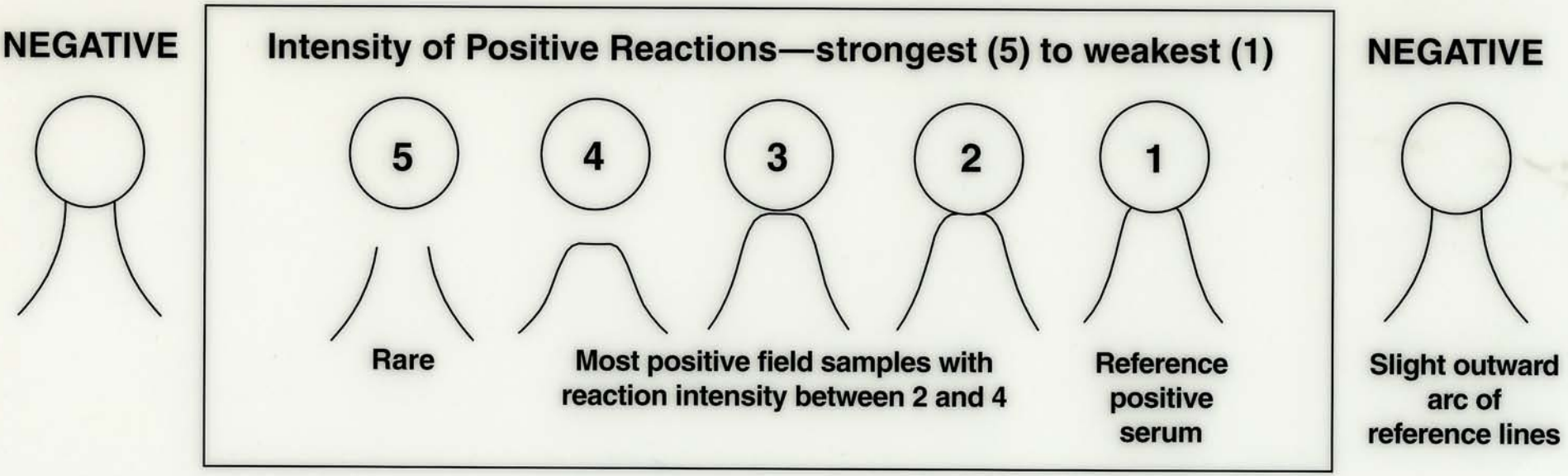
Interpret accurately.



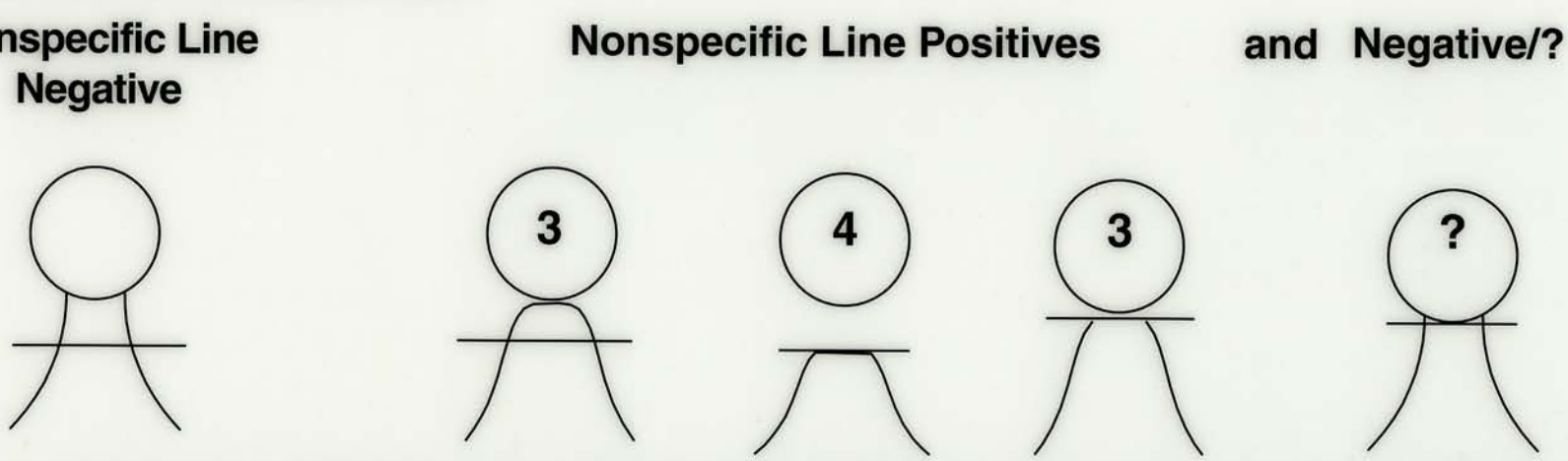
Samples 2 and 3 (taken 21 and 29 days after infection) are positive.

To contact the authors of this Program Aid for more information, please see reverse.

Range of Reactions To Expect in the AGID (Coggins) Test



ADDITIONAL POSSIBILITIES:



Notes

Interpret all samples according to the termination of control reference positive lines.

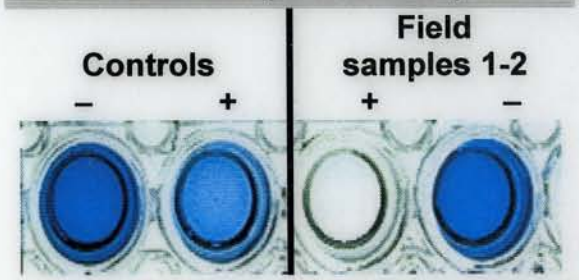
When in doubt, or if qualified readers disagree, samples should be forwarded to a reference laboratory. Supplemental testing and/or an additional sample, collected at a later date, may be required to accurately determine the status of the horse.

If you get a second sample, test both at the same time and compare reactions.

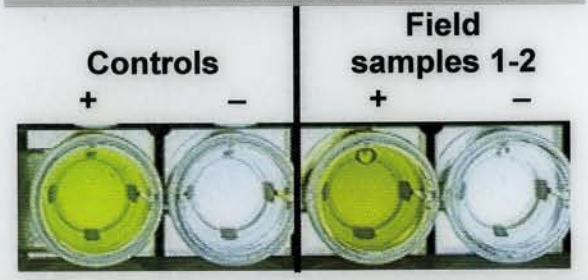
To contact the authors of this Program Aid for more information, please see reverse.

ELISA Test Formats for EIA and Expected Results

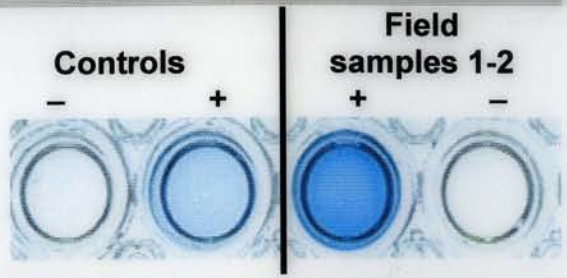
CELISA (IDEXX Labs)



SA-ELISA II (Centaur, Inc.)



Vira-CHEK (Synbiotics Corp.)



Optical density (OD) at the appropriate wavelength in a spectrophotometer (spec)

1.14	0.57	0.09	1.08	1.24	0.04	> 2	0.04	0.04	0.17	0.57	0.04

Notes

- Competition against monoclonal on plate.
- Reject hemolyzed samples.
- 0.1 mL sample.
- Deionized water washes.
- **Compare reactions against POS control (less than or equal color is positive).**
- Visual or spec reading (650 nm).

- Avoid cross contamination; hold plate properly.
- 0.05 mL sample.
- Use detergent wash solution; avoid bubbles.
- Add stop solution.
- **Compare reactions against NEG control (0.05 OD above the NEG control is positive).**
- Visual or spec reading (450 nm).

- Add conjugate first.
- 0.05 mL sample.
- First washes with detergent; avoid bubbles.
- Final deionized or distilled water washes.
- **Compare reactions against POS control (more than or equal color is positive).**
- Visual or spec reading (630 nm).

Additional comments

False-negative results occur with lower frequency in ELISA tests than in AGID tests. False-positive results occur with higher frequency in ELISA tests than in AGID tests. Rarely are false-positive ELISA samples positive in more than one ELISA test format. All samples positive in all three ELISA formats investigated until now have been positive for antibodies against EIAV in immunoblot tests; the vast majority will be interpreted by all diagnosticians as positive by AGID. One added benefit of ELISA testing is that objective measurement of the color in the wells can be done by spectrophotometer. That removes the possible error because of differences in subjective color discrimination between individuals and gives an objective recording of the results that may be useful in laboratory reproducibility and oversight.

The spectrophotometer is very useful for samples that develop colors close to the cut-off value for positive. If such samples are from EIAV-exposed horses and not just false-positive reactions, they probably are from horses recently exposed to EIAV. In samples collected from newly infected animals, equivocal results may be seen for a very short time in each of the official test formats including the AGID test. When the EIA test results are equivocal, preliminary findings must be reported to the state regulatory officials. A second sample needs to be collected from the horse and tested to determine the true infection status. In most cases, the infected horse will test unequivocally positive in all ELISA and AGID tests 14-28 day after the first equivocal results are observed. The vast majority of horses infected with EIAV yield positive results on all EIA tests within 45 days of exposure to the virus.

For more information, please see reverse.

Always read the instructions and follow the protocols exactly!

Timothy R. Cordes, D.V.M.
Senior Staff Veterinarian, Equine Programs, National Animal Health Program Staff, Veterinary Services, Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), Riverdale, MD.
(301) 734–3279 or
Timothy.R.Cordes@aphis.usda.gov

Charles J. Issel, D.V.M., Ph.D.
Wright–Markey Chair of Equine Infectious Diseases, University of Kentucky, Gluck Equine Research Center, Lexington, KY.
(859) 257–1710 or cissel@uky.edu

Eileen N. Ostlund, D.V.M., Ph.D.
Section Head, Equine and Ovine Viruses, Diagnostic Virology Laboratory, National Veterinary Services Laboratories of USDA–APHIS–Veterinary Services, Ames, IA.
(515) 663–7551 or
Eileen.N.Ostlund@aphis.usda.gov

Beverly J. Schmitt, D.V.M., M.S.
Chief, Diagnostic Virology Laboratory, National Veterinary Services Laboratories of USDA–APHIS–Veterinary Services, Ames, IA.
(515) 663–7551 or
Beverly.J.Schmitt@aphis.usda.gov

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