Supplementary materials for Journal of Wildlife Diseases DOI: 10.7589/2018-01-015: María J. Forzán, Randall W. Renshaw, Elizabeth M. Bunting, Elizabeth Buckles, Joseph Okoniewski, Kevin Hynes, Melissa Laverack, Melissa Fadden, Akbar Dastjerdi, Krysten Schuler, Edward J. Dubovi. **A NOVEL ORTHOREOVIRUS ASSOCIATED WITH EPIZOOTIC NECROTIZING ENTERITIS AND SPLENIC NECROSIS IN AMERICAN CROWS (*CORVUS BRACHYRHYNCHOS*)**

Supplementary File 1

ISH Protocol Using ACD Reagents (Red modifications)

1. Cut tissue into 5µm onto charged slides, and then allow them to dry upright overnight. For optimal results tissue should be cut fresh or kept in -80°C until needed.
2. Heat treat the slides for 4**0 minutes** at 60°C (slide warmer).
3. Deparafinize slides: 2 changes of xylene **(5 minutes** each), 2 changes of 100% ethanol (**3 minutes** each)
4. Allow the tissue to dry completely.
5. Prepare 700mL of Target Retrieval Reagent (70mL of concentrate in 630mL of water). Bring the solution to a boil in a beaker covered with foil on a hot plate. NOTE: don't allow the solution to boil for more than **15 minutes** before you add the slides.
6. Add 5-8 drops of hydrogen peroxide to each slide making sure to cover the tissue and incubate at RT for **10 minutes**. The solution is part of the ACDBio kit – 8 drops are needed if the slide contains numerous tissues.
7. Flick the solution into a waste container (or just add distilled water) and wash the slides by moving them up and down in the distilled water 3-5 times. *[Do not let the slides dry out from this point on]*
8. Repeat the wash with fresh water.
9. Add the slides in a gray slide rack to the antigen retrieval solution boil for **15 minutes**. Watch the slides to make sure the solution doesn't boil over. *[Remove the upper handle of the grey rack, as it can melt in the boiling water]* Note: cut to 10 min for cell pellets.
10. Remove the slides from the solution and place them in water. Wash the slides by moving them up and down in the water 3-5 times.
11. Repeat the wash with fresh water.
12. Wash slides with fresh 100% ethanol and allow them to dry at RT.
13. Draw around the slides with a hydrophobic barrier pen and allow the barrier to dry. NOTE: you can stop at this point.
14. Warm probes and Wash Buffer concentrate to 40°C.
15. Add 50mL of water to a piece of filter paper at the bottom of the humidity tray.
16. Add 5 drops of Protease to each slide making sure to cover the tissue. Incubate in the oven at 40°C for **30 minutes**. Note: cut to 15 min for cell pellets.

17. Flick the solution into a waste container and wash the slides by moving them up and down in the water 3-5 times.
18. Tap to dry each slide and add 4 drops of probe to the tissue outlined by hydrophobic pen. Only if necessary spread probe with sterile toothpick or pipette tip – this is, however, usually unnecessary if there is enough liquid on the slide surface so the probe spreads itself.
19. Incubate in the oven at 40^{o}C for **2 hours**.
20. Prepare 1X wash buffer (60mL of concentrate in 2.94L of water).
21. Allow AMP 1-6 reagents warm to RT.
22. Flick the solution into a waste container and wash in 1X wash buffer for **2 minutes** at RT. Agitate slides by moving them up and down a few times.
23. Repeat with fresh 1X wash buffer (**2 min**).
24. Tap to dry each slide and add 4 drops of AMP 1 to the tissue outlined by hydrophobic pen.
25. Incubate in the oven at 40^{o}C for **30 minutes**.
26. Flick the solution into a waste container and wash in 1X wash buffer for 2 minutes at RT. Agitate slides by moving them up and down a few times.
27. Repeat with fresh 1X wash buffer (**2 min)**.
28. Tap to dry each slide and add 4 drops of AMP 2 to the tissue outlined by hydrophobic pen.
29. Incubate in the oven at 40^{o}C for **15 minutes**.
30. Flick the solution into a waste container and wash in 1X wash buffer for 2 minutes at RT. Agitate slides by moving them up and down a few times.
31. Repeat with fresh 1X wash buffer.
32. Tap to dry each slide and add 4 drops of AMP 3 to the tissue outlined by hydrophobic pen.
33. Incubate in the oven at 40^{o}C for **30 minutes**.
34. Flick the solution into a waste container and wash in 1X wash buffer for 2 minutes at RT. Agitate slides by moving them up and down a few times.
35. Repeat with fresh 1X wash buffer (**2 min)**.
36. Tap to dry each slide and add 4 drops of AMP 4 to the tissue outlined by hydrophobic pen.
37. Incubate in the oven at 40^{o}C for **15 minutes**.
38. Flick the solution into a waste container and wash in 1X wash buffer for 2 minutes at RT. Agitate slides by moving them up and down a few times.
39. Repeat with fresh 1X wash buffer.
40. Tap to dry each slide and add 4 drops of AMP 5 to the tissue outlined by hydrophobic pen.
41. Incubate on the bench at RT for **30 minutes**. Note: cut to 15, 10 or even 5 min to reduce overstaining.
42. Flick the solution into a waste container and wash in 1X wash buffer for 2 minutes at RT. Agitate slides by moving them up and down a few times.
43. Repeat with fresh 1X wash buffer (**2 min)**.
44. Tap to dry each slide and add 4 drops of AMP 6 to the tissue outlined by hydrophobic pen.
45. Incubate on the bench at RT for **15 minutes**.
46. Flick the solution into a waste container and wash in 1X wash buffer for 2 minutes at RT. Agitate slides by moving them up and down a few times.
47. Repeat with fresh 1X wash buffer (**2 min)**.
48. Prepare Red solution. Centrifuge Red B tube. Dilute Red B into Red A (1:60). 120ul of Red solution is adequate for all but the largest of tissue sections. Mix immediately prior to use and then let sit for **2 min** before using it.
49. Tap to dry each slide and add 120µL of Red mix to each slide in the HybEZ slide rack/control tray.

50. Incubate with Red solution at room temperature for **5 minutes**. Note: cut to 2 min for cell pellets.
51. Wash the slides by moving them up and down in the water 3-5 times. Repeat with clean water.
52. Counter stain in Hematoxylin for **2 minute**.
53. Move the slides quickly into water and bring them to the sink. Dump the water and allow the slides to run under tap water for **3 minutes**.
54. Dehydrate the slides by placing them on the slide warmer (60C) for **15 min** or until dry. Cover the slides with the tray lid to prevent additional light exposure.
    a. **Do not dehydrate slides with ethanol!**
55. Place in Histoclear solution #1 for **2 min**, and then histoclear #2 (**2 min)** and #3 (**2 min)**.
56. Mount coverslips using Ecomount.

NOTE: To stain 10 slides you need approximately 20 µL of red B + 1180 µL of red A

Supplementary Table 1

American crow, *Corvus brachyrhynchos*, number of carcasses (n) submitted to the New York State Wildlife Health Program during 2001-2015 and diagnosed with reovirosis. Case definition: Confirmed (histopathological necrosis in spleen and/or intestine and either positive reovirus isolation from fresh tissue or a positive *in situ* hybridization [ISH] staining in the lesions), Probable (gross splenic or intestinal necrosis without histopathological confirmation but with positive reovirus isolation, or histopathological evidence of necrosis in spleen and/or intestine without reovirus isolation or ISH) or Suspect (gross necrosis in spleen and/or intestine without confirmatory virus isolation, histopathology or ISH). Months with 0 reovirosis cases are excluded.

Year	Month	Reovirosis status	n
2001	December	Probable	1
2001	December	Suspect	17
2002	January	Probable	6
2002	January	Suspect	32
2002	February	Suspect	16
2002	March	Suspect	7
2002	April	Suspect	5
2002	December	Suspect	4
2003	January	Suspect	17
2003	February	Suspect	14
2003	March	Suspect	15
2003	April	Suspect	1
2003	October	Suspect	1
2003	December	Suspect	4
2004	January	Suspect	4
2004	February	Suspect	3
2004	October	Suspect	1
2005	January	Suspect	6
2005	February	Suspect	3
2005	March	Suspect	3
2005	April	Suspect	4
2005	October	Suspect	1
2005	December	Suspect	47
2006	February	Suspect	10
2006	March	Suspect	1
2006	May	Suspect	1
2006	June	Suspect	1
2006	September	Suspect	1
2006	December	Suspect	5

2007	January	Suspect	4
2007	February	Suspect	4
2007	November	Suspect	2
2007	December	Probable	5
2007	December	Suspect	65
2008	January	Probable	8
2008	January	Suspect	60
2008	February	Suspect	1
2008	March	Suspect	4
2008	April	Suspect	1
2008	May	Suspect	1
2008	August	Probable	2
2008	October	Suspect	1
2008	November	Suspect	3
2008	December	Suspect	3
2009	January	Suspect	23
2009	February	Suspect	17
2009	March	Suspect	7
2010	January	Confirmed	2
2010	January	Suspect	14
2010	February	Confirmed	1
2010	February	Suspect	17
2010	March	Suspect	2
2011	January	Confirmed	23
2011	January	Probable	2
2011	February	Confirmed	1
2011	February	Suspect	5
2011	March	Confirmed	1
2011	July	Probable	1
2012	January	Probable	2
2012	March	Probable	1
2013	January	Confirmed	2
2013	January	Probable	1
2013	January	Suspect	4
2013	February	Confirmed	1
2014	March	Confirmed	1
2014	September	Confirmed	1
2015	May	Confirmed	1