	NATIONAL CENTRE FOR ANIMAL HEALTH **LABORATORY SERVICES UNIT**	**Second edition**
	STANDARD OPERATING PROCEDURE	**Version 2018.1**
	RAPID TESTS	

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TITLE: Rapid Antigen Detection for Avian Influenza Type A

PREPARED BY: Virology section

REVISED BY: Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION
Avian influenza (AI) is a devastating disease to many avian species. Currently, a circulating highly pathogenic, zoonotic H5N1 virus (HPAI H5N1) and H7N9 (earlier considered low pathogenic) make it critical to be familiar with the signs and laboratory procedures to diagnose the disease. Rapid diagnosis ensures that control measures are promptly enacted.

2. PRINCIPLES
Quicking[a] Avian Influenza Virus Antigen Test is based on sandwich lateral flow immunochromatographic assay for qualitative detection of avian influenza virus. Each box contains 10x foil pouches, each containing one cassette, one pipette and a desiccant, 10x assay buffer tubes (0.8mL each), 10x swab sticks and a product manual. The simple assay is based on rapid immuno migration technology, where antibodies in the strip combine with influenza A nucleoprotein (antigen) in the sample. The antigen/antibody complex combines with another antibody further along the strip, and the accumulated complexes form a coloured line. The assay takes about 10 minutes to run. The test device has a testing window. The testing window has an invisible T (test) zone and C (control) zone. When sample is applied into the sample hole on the device, the liquid will laterally flow on the surface of the test strip. If there are enough AIV antigens in the sample, a visible T band will appear. The C band should always appear after a sample is applied, indicating a valid result. By this means, the device can accurately indicate the presence of Avian Influenza Virus antigen in the sample.

3. APPLICATION
This test kit is used for rapid detection of Avian Influenza type A antigen**.**

4. OBJECTIVE
To outline the procedure to for screening of samples for Avian Influenza Type A antigen.

5. TEST KITS, REAGENTS, SOLUTION AND BUFFER
5.1 10×foil pouches, each containing one cassette, one pipette and a desiccant
5.2 10×assay buffer tubes (0.8 mL each)
5.3 10×swab sticks
5.4 Product Manual

6. TEST PROCEDURE

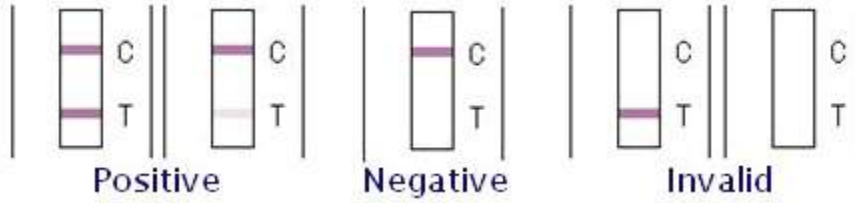
6.1 Insert the swab stick into bird's cloaca or di the secretions. Please make the swab wet sufficiently.
6.2 Insert the wet swab into the provided assay buffer tube. Agitate it to assure good sample extraction.
6.3 Take out the cassette from the foil pouch and place it horizontally.
6.4 Gradually drip 3 drops of sample extraction into the sample loading well
6.5 Interpret the result in 5-10 minutes. Result after 10 minutes is considered as invalid.
6.6 Follow the test procedures as described in the guide protocol.

a. Where ever possible do not pool unlike samples i.e. do not mix tracheal swab with cloacal swab for testing. This might give false positive results.
b. In pooling samples, take approximately 5 samples to exercise economy.
c. Timing is an important determining factor for reading accurate results. Use stop watch to keep the timing while performing the test either in the lab or field.
d. As far as possible perform the test in the field using this kit while the sample is still fresh.
e. For testing in the laboratory, ensure proper refrigeration of the samples in the cool pack (8-10^{0}C). Use nutrient broth or viral transport media (VTM) as transport media for samples.

7. RESULT INTERPRETATION

7.1 Positive: After the test is completed appearance of colour band on both C and T bands are observed, no matter T band is clear or vague.
7.2 Negative: Only clear C band appears.
7.3 Invalid: If the C band does not appear, irrespective of the appearance or non-appearance of T band the test is considered invalid.
7.4 Results are reported as positive or negative
7.5 All results should be considered in conjunction with other clinical information available from veterinarian. For further confirmation, it is suggested to choose other method such as polymerase chain reaction (PCR)

Below is the pictorial representation of test result



8. WASTE DISPOSAL

8.1 Dispose the tests materials safely
8.2 Ensure that the test materials (swabs, pipettes, vials, containers etc.) are properly packed and are brought back to the laboratory for proper disinfection by autoclaving and disposed of safely in the pit.

8.3 Alternatively, the test materials and samples should be disinfected in 2% Virkon-S and autoclaved

9. RISK ASSESSMENT

9.1 Use full personal protective equipment (PPE) for suspect cases
9.2 While doing post mortem, maximum precautions are to be taken (this is a technical discipline to be followed every time).
9.3 All specimens from suspected cases should be considered potentially infectious and ensure personal safety precautions

10. TROUBLESHOOTING

10.1 Viscous sample preparation can prevent lateral flow of sample and reaction leading to delayed reading time.
10.2 Avoid bubble formation while loading sample on cassette

11. REFERENCES

Avian Influenza Virus Ag Test, Cat No.: W81033, Quicking Biotech Co., Ltd., Shanghai, China

TITLE: Rapid detection of Influenza A H5 Antigen

PREPARED BY: Virology section

REVISED BY: Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION
Avian influenza (AI) is a devastating disease to many avian species. Currently, a circulating highly pathogenic, zoonotic H5N1 virus (HPAI H5N1) and H7N9 (earlier considered low pathogenic) make it critical to be familiar with the signs and laboratory procedures to diagnose the disease. It is essential to ensure the involvement of sub type H5 in HPAI.
Rapid diagnosis ensures that control measures are promptly enacted.

2. PRINCIPLES
Avian Influenza Virus H5 Antigen Rapid Test is a sandwich lateral flow immuno-chromatographic assay (Quicking[a]) for the qualitative detection of avian influenza virus H5 subtype (AIV H5 Ag) in avian secretions.

3. APPLICATION
This tests is used for rapid detection of Avian Influenza sub-type H5 antigen**.**

4. OBJECTIVE
To outline the procedure for detection of Avian Influenza A sub-type H5 antigen

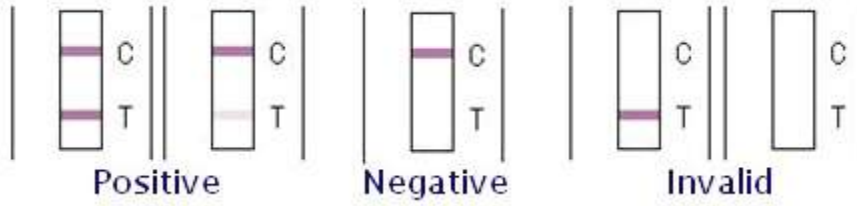
5. TEST KITS, REAGENTS, SOLUTION AND BUFFER
5.1 10×foil pouches, each containing one cassette, one pipette and a desiccant
5.2 10×assay buffer tubes (0.8 mL each)
5.3 10×swab sticks
5.4 Product Manual

6. PROCEDURE
6.1 Insert the swab stick into bird's cloacae the secretions. Please make the swab wet sufficiently.
6.2 Insert the wet swab into the provided assay buffer tube. Agitate it to assure good sample extraction.
6.3 Take out the cassette from the foil pouch and place it horizontally.
6.4 Gradually drip 3 drops of sample extraction into the sa
6.5 Interpret the result in 5-10 minutes. Result after 10 minutes is considered as invalid.

7. RESULT INTERPRETATION

7.1 Positive: After the test is completed appearance of colour band on both C and T bands are observed, no matter T band is clear or vague.
7.2 Negative: Only clear C band appears.
7.3 Invalid: If the C band does not appear, irrespective of the appearance or non-appearance of T band the test is considered invalid.
7.4 Results are reported as positive or negative
7.5 All results should be considered in conjunction with other clinical information available from veterinarian. For further confirmation, it is suggested to choose other method such as polymerase chain reaction (PCR)

Below is the pictorial representation of test result



8. WASTE DISPOSAL

8.1 Dispose the tests materials safely
8.2 Ensure that the test materials (swabs, pipettes, vials, containers etc.) are properly packed and are brought back to the laboratory for proper disinfection by autoclaving and disposed of safely in the pit.
8.3 Alternatively, the test materials and samples should be disinfected in 2% Virkon-S and autoclaved

9. RISK ASSESSMENT

9.1 Use full personal protective equipment (PPE) for suspect cases
9.2 While doing post mortem, maximum precautions are to be taken (this is a technical discipline to be followed every time).
9.3 All specimens from suspected cases should be considered potentially infectious and ensure personal safety precautions

10. TROUBLESHOOTING

10.1 Viscous sample preparation can prevent lateral flow of sample and reaction leading to delayed reading time.
10.2 Avoid bubble formation while loading sample on cassette

11. REFERENCES

Avian Influenza Virus sub-type H5 Ag Test, Cat No.: W81033, Quicking Biotech Co., Ltd., Shanghai, China

TITLE: Rapid antigen detection for Rabies

PREPARED BY: Virology section

REVISED BY: Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION
The Anigen Rapid Rabies Ag Test Kit is a chromatographic immunoassay for the qualitative detection of Rabies virus antigen in canine, bov brain homogenates.

2. PRINCIPLES
The test is based on the sandwich lateral flow immunochromatographic assay.

3. APPLICATION
The test is used for rapid detection of Rabies virus antigen.

4. OBJECTIVE
To outline the procedure for rapid detection of Rabies virus antigen

5. APPARATUS/TEST KIT/BUFFER
5.1 Ten (10) Antigen Rapid Rabies Ag test devices
5.2 Ten (10) Assay diluents (1ml)
5.3 Ten (10) sample collection swabs
5.4 Ten (10) disposable droppers
5.5 One(1) instruction for use

6. PROCEDURE
6.1 Collect the samples from saliva, and brain homogenates using the swab.

6.2 Insert the swab into the specimen tube containing 1ml of assay diluent.

6.3 Mix the swab samples with assay diluent to extract well.

6.4 Remove the test device from the foil pouch, and place it on a flat and dry surface.

6.5 Using the disposable dropper provided, take the samples from extracted and mixed specimens in the tube.

6.6 Add four (4) drops into the sample hole using the disposable dropper.

6.7 As the test begins to work, you will see purple color move across the result window in the center of the test device. If the migration has not appeared after 1 minute, add one more drop of the mixed assay diluent to the sample well.

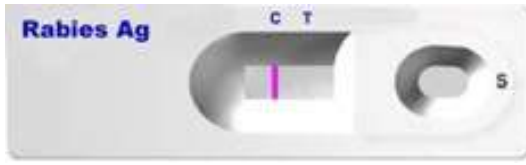
6.8 Interpret test results at 5 ~ 10 minutes. Do not decide after 10 minutes.

7. RESULT INTERPRETATION

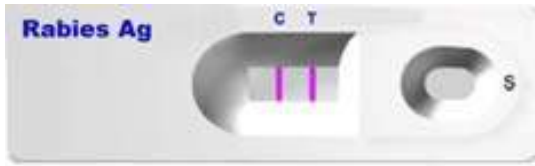
Interpret test results at 5 ~ 10 minutes. Do not decide after 10 minutes.

A color band will appear in the left section of the result window to show that the test is working properly. This band is the control band. The right section of the result window indicates the test results. If another color band appears in the right section of the result window. This band is the test band.

Negative result: The presence of only one band (C) within the result window indicates a negative result.



Positive result: The presence of two color bands ("T" and "C" within) the result window, no matter which band appears first indicates a positive result.



After the completion of Laboratory test the result based on the interim format of NCAH may be sent to the concerned In-Charges. Results are reported either as positive or negative.

8. WASTE DISPOSAL

8.1 Dispose the tests materials safely

8.2 Ensure that the test materials (Brain) are properly packed and are brought back to the laboratory for proper disinfection by autoclaving and disposed of safely in the pit.

8.3 Alternatively, the test materials and samples should be disinfected in 2% Virkon-S and autoclaved

9. RISK ASSESSMENT

9.1 Do not open or remove test kit from their individually sealed pouches until immediately before their use.

9.2 Do not use the test kit if the pouch is damaged or the seal is broken.

9.3 Do not reuse test kit.

9.4 All reagents must be at room temperature before running the assay.

9.5 Do not use reagents beyond the stated expiration date marked on the label.

9.6 The kit can be stored at room temperature (2~30) or refrigerated.

9.7 **DO NOT FREEZE**. Do not store the test kit in direct sunlight.

9.8 Although the Antigen Rapid Rabies Ag Test kit is very accurate in detecting

Rabies virus antigen, a low incidence of false results can occur. Other clinically available tests are required if questionable results are obtained.

9.9 As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the veterinarian after all clinical and laboratory findings have been evaluated.

10. TROUBLESHOOTING

10.1 Brain sample must be preserved in 50% Glycerine saline of pH 7.4

10.2 Thick smear may give you wrong interpretation.

11. REFERENCES

The Anigen Rapid Rabies Ag Test BioNote, Inc. Seogu-dong, Hwaseong-si, Gyeonggi-do, Korea

Anonymous (2001). In: Rabies Diagnosis Manual. (Edited by King, A. Bishop, G. Bingham, J., Wandeler, A. and Barrat, J.) Southern and Eastern African Rabies Group, 22 pp

TITLE: Rapid Antigen Detection for Infectious Bursal Disease

PREPARED BY: Virology section

REVISED BY: Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION
Quicking Infectious Bursal Disease virus antigen rapid test kit (cat no. W81057) is a sandwich lateral flow immunochromatography assay for detection of Infectious bursal disease virus in avian secretions.

2. PRINCIPLES
Quicking infectious Bursal Disease Virus AG rapid antigen test is based on sandwich lateral flow immunochromatographic assay. The test device has a testing window. The testing window has an invisible T (test) zone and C (control) zone. When sample is applied into the sample hole on the device, the liquid will laterally flow on the surface of the test strip. If there are enough IBDV antigens in the sample, a visible T band will appear. The C band should always appear after a sample is applied, indicating a valid result. This means, the device can accurately indicate the presence of infectious Bursal Disease Virus antigen in the sample

3. APPLICATION
This test is used for the rapid detection of infectious Bursal Disease Virus antigen in the sample.

4. OBJECTIVE
To outline the procedure for rapid diagnosis of infectious Bursal Disease Virus antigen in the sample

5. TEST KITS, REAGENTS, SOLUTION AND BUFFER
5.1 10 × foil pouches, each containing one cassette, one pipette and a desiccant
5.2 10 × assay buffer tubes (0.5 mL each)
5.3 10 × swab sticks
5.4 Product Manual

6. TEST PROCEDURE
6.1 Insert the swab stick into bird's cloaca from slaughtered Bursicious poultry to collect the secretions. Please make the swab wet sufficiently.
6.2 Insert the wet swab into the provided assay buffer tube. Agitate it to assure good sample extraction.
6.3 Take out the cassette from the foil pouch and place it horizontally.
6.4 Gradually drip 3 drops of sample extraction into the sample loading well

6.5 Interpret the result in 5-10 minutes. Result after 10 minutes is considered as invalid.

6.6 Follow the test procedures as described in the guide protocol.

a. Where ever possible do not pool unlike samples i.e. do not mix tracheal swab with cloacal swab for testing. This might give false positive results.
b. In pooling samples, take approximately 5 samples to exercise economy.
c. Timing is an important determining factor for reading accurate results. Use stop watch to keep the timing while performing the test either in the lab or field.
d. As far as possible perform the test in the field using this kit while the sample is still fresh.
e. For testing in the laboratory, ensure proper refrigeration of the samples in the cool pack (8-10^{0}C). Use nutrient broth or viral transport media (VTM) as transport media for samples.

7. RESULT INTERPRETATION

7.1 Positive: After the test is completed appearance of colour band on both C and T bands are observed, no matter T band is clear or vague.

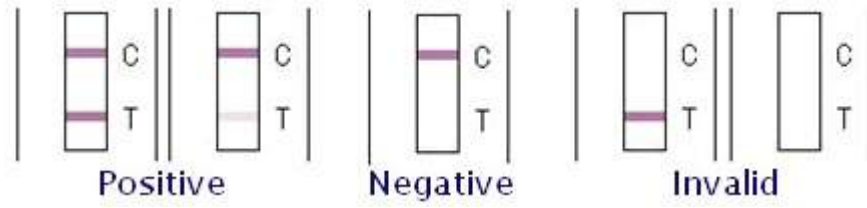
7.2 Negative: Only clear C band appears.

7.3 Invalid: If the C band does not appear, irrespective of the appearance or non-appearance of T band the test is considered invalid.

7.4 Results are reported as positive or negative

7.5 All results should be considered in conjunction with other clinical information available from veterinarian. For further confirmation, it is suggested to choose other method such as polymerase chain reaction (PCR)

Below is the pictorial representation of test result



8. WASTE DISPOSAL

8.1 Dispose the tests materials safely

8.2 Ensure that the test materials (swabs, pipettes, vials, containers etc.) are properly packed and are brought back to the laboratory for proper disinfection by autoclaving and disposed of safely in the pit.

8.3 Alternatively, the test materials and samples should be disinfected in 2% Virkon-S and autoclaved

9. RISK ASSESSMENT

9.1 Use full personal protective equipment (PPE) for suspect cases

9.2 While doing post mortem, maximum precautions are to be taken (this is a technical discipline to be followed every time).

9.3 All specimens from suspected cases should be considered potentially infectious and ensure personal safety precautions

10. TROUBLESHOOTING

10.1 Viscous sample preparation can prevent lateral flow of sample and reaction leading to delayed reading time.

10.2 Avoid bubble formation while loading sample on cassette

11. REFERENCES

Infectious bursal disease virus Ag test, cat no. W81057 edition 2014 06/11

TITLE: Rapid Antigen test for Newcastle Disease

PREPARED BY: Virology section

REVISED BY: Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION
Quicking Newcastle Disease Rapid test is based on sandwich lateral flow immunochromatographic assay (Cat No.: W81043) for detecting the antigen for NCD virus in serum or secretions of poultry samples.

2. PRINCIPLES
Quicking Newcastle Disease Rapid test is based on sandwich lateral flow immunochromatographic assay. The test device has a testing window. The testing window has an invisible T (test) zone and C (control) zone. When sample is applied into the sample hole on the device, the liquid will laterally flow on the surface of the test strip. If there are enough NCDV antigens in the sample, a visible T band will appear. The C band should always appear after a sample is applied, indicating a valid result. This means, the device can accurately indicate the presence of Newcastle Disease Virus antigen in the sample

3. APPLICATION
This tests is used for rapid detection of Newcastle Disease Virus antigen in the sample.

4. OBJECTIVE
The test is performed to diagnose Newcastle Disease Virus antigen in the sample

5. TEST KITS, REAGENTS, SOLUTION AND BUFFER
5.1 10×foil pouches, each containing one cassette, one pipette and a desiccant
5.2 10×assay buffer tubes (0.5 mL each)
5.3 10×swab sticks
5.4 Product Manual

6. TEST PROCEDURE
6.1 Insert the swab stick into bird's eyes, trache
Please make the swab wet sufficiently.
6.2 Insert the wet swab into the provided assay buffer tube. Agitate it to assure good sample extraction.
6.3 If using serum sample, please do a dilution of 1:2 with the provided assay buffer
6.4 Take out the cassette from the foil pouch and place it horizontally.
6.5 Gradually drip 3 drops of sample extraction into the sample loading well

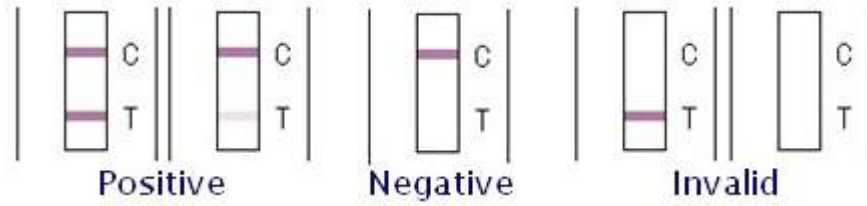
6.6 Interpret the result in 5-10 minutes. Result after 10 minutes is considered as invalid.

a. Where ever possible do not pool unlike samples i.e. do not mix tracheal swab with cloacal swab for testing. This might give false positive results.
b. In pooling samples, take approximately 5 samples to exercise economy.
c. Timing is an important determining factor for reading accurate results. Use stop watch to keep the timing while performing the test either in the lab or field.
d. As far as possible perform the test in the field using this kit while the sample is still fresh.
e. For testing in the laboratory, ensure proper refrigeration of the samples in the cool pack (8-10^{0}C). Use nutrient broth or viral transport media (VTM) as transport media for samples.

7. RESULT INTERPRETATION

7.1 Positive: After the test is completed appearance of colour band on both C and T bands are observed, no matter T band is clear or vague.
7.2 Negative: Only clear C band appears.
7.3 Invalid: If the C band does not appear, irrespective of the appearance or non-appearance of T band the test is considered invalid.
7.4 Results are reported as positive or negative
7.5 All results should be considered in conjunction with other clinical information available from veterinarian. For further confirmation, it is suggested to choose other method such as polymerase chain reaction (PCR)

Below is the pictorial representation of test result



8. WASTE DISPOSAL

8.1 Dispose the tests materials safely
8.2 Ensure that the test materials (swabs, pipettes, vials, containers etc.) are properly packed and are brought back to the laboratory for proper disinfection by autoclaving and disposed of safely in the pit.
8.3 Alternatively, the test materials and samples should be disinfected in 2% Virkon-S and autoclaved

9. RISK ASSESSMENT

9.1 Use full personal protective equipment (PPE) for suspect cases

9.2 While doing post mortem, maximum precautions are to be taken (this is a technical discipline to be followed every time).

9.3 All specimens from suspected cases should be considered potentially infectious and ensure personal safety precautions

10. TROUBLESHOOTING

10.1 Viscous sample preparation can prevent lateral flow of sample and reaction leading to delayed reading time.

10.2 Avoid bubble formation while loading sample on cassette

11. REFERENCES

Newcastle disease virus Ag test, cat no. W81043 edition 2014 06/11

TITLE: Rapid Antigen detection for PPR virus

PREPARED BY: Virology section

REVISED BY: Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION
Peste des petits ruminants virus (PPRV), also known as goat plague, is highly contagious and infects small ruminants such as sheep and goats. Cattle and pigs can be infected but don't develop any clinical signs. It is a virus belonging to *Paramyxoviridae* family, genus *Morbillivirus* and has 4 lineages. It is enveloped and has a capsid that contains a single-stranded RNA genome.

2. PRINCIPLES
The PESTE-TEST is based on sandwich lateral flow immunochromatographic assay for qualitative detection of PPR virus.

3. APPLICATION
This tests is used for rapid detection of PPR virus antigen in the sample.

4. OBJECTIVE
To describe the procedure for rapid detection of PPR virus antigen in the sample.

5. TEST KITS, REAGENTS, SOLUTION AND BUFFER
The kit contains 25 lateral flow devices with all the necessary buffers and sample tubes to test 25 field samples for PPR antigen.

6. TEST PROCEDURE
Ensure buffer and test device are at ambient temperature before beginning.

6.1 Take one of the sample tubes provided and add 30 drops of the provided buffer from the dropper bottle.

6.2 Take one of the included swabs and use it to swab the inside of the lower eyelid, or the inside of the nose, of the suspected animal. Eye swabs are generally preferred to nasal swabs as they contain less mucus, but either will work.

6.3 Place the cotton-bud end of the swab into the buffer in the sample tube and agitate it in the buffer for 5-10 seconds.

6.4 Remove the swab from the sample tube and discard.

6.5 Unwrap one test device (LFD) and lay it on a flat surface out of direct sunlight. Using one of the included pastettes, transfer 4 drops from the sample tube to the sample application well on the test device.

6.6 Leaving the test device flat, allow the test to develop for up to 20 minutes (if the test is clearly positive before 20 minutes, further incubation is not required

7. RESULT INTERPRETATION

A band appearing at the 'T' (test) position is positive res

8. WASTE DISPOSAL

8.1 Dispose the tests materials safely

8.2 Ensure that the test materials (swabs, pipettes, vials, containers etc.) are properly packed and are brought back to the laboratory for proper disinfection by autoclaving and disposed of safely in the pit.

8.3 Alternatively, the test materials and samples should be disinfected in 2% Virkon-S and autoclaved

9. RISK ASSESSMENT

All specimens from suspected cases should be considered potentially infectious and dispose of properly.

10. TROUBLESHOOTING

10.1 Viscous sample preparation can prevent lateral flow of sample and reaction leading to delayed reading time.

10.2 Avoid bubble formation while loading sample on cassette

11. REFERENCES

PESTE-TEST version 2.0 April 2014

TITLE: Rapid detection of Canine Distemper Virus Antigen

PREPARED BY: Serology & Virology section

REVISED BY: Serology & Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION
Canine Distemper Virus Ag Test is a sandwich lateral flow immunochromatographic assay for the qualitative detection of ca secretions or serum.

2. TEST PRINCIPLES
The test is based on the Sandwich lateral flow immunochromatographic assay

3. APPLICATION
The test is used to diagnose Canine Distemper virus infection in dogs.

4. OBJECTIVE
To describe the procedure for detection of CDV antigen

5. APPARATUS/TEST KIT/REAGENTS
5.1 Foil pouches, each containing one cassette, one pipette and a desiccant
5.2 Assay buffer tubes
5.3 Swab sticks
5.4 Product Manual

6. PROCEDURE
6.1 Allow all kit components and specimen to reach room temperature prior to testing.

6.2 Collect 5 of sample using capillary tube (point line), and then add the specimen into the diluent tube. A dark color score line on the capillary tube is the indicator line for 5 .

6.3 Remove the test kit from the foil pouch prior to use.

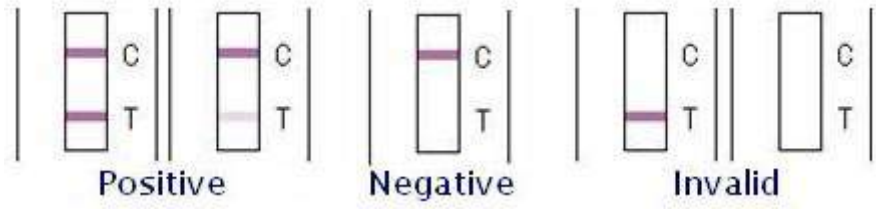
6.4 Using the disposable dropper provided, take the samples from the mixed assay diluent in the tube.

6.5 Add four (4) drops into the sample hole using the disposable dropper slowly drop by drop.

6.6 Interpret the test results at 10 minutes. Do not interpret after 20 minutes.

7. RESULT INTERPRETATION and REPORTING

Positive: The presence of both C band and T band, no matter T band is clear or vague.
Negative: Only clear C band appears.
Invalid: No colored band appears in C zone, no matter whether T band appears.



8. WASTE DISPOSAL
Dispose in proper disinfectant.

9. RISK ASSESSMENT
All specimens should be handled as being potentially infectious.

10. TROUBLESHOOTING
NA

11. REFERENCES

Anigen Rapid CDV Ag Test kit, Doc No.1103-10E Apr 17, 2017

TITLE: Rapid detection of Canine parvo virus antigen

PREPARED BY: Serology & Virology section

REVISED BY: Serology & Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION

Canine Parvovirus (CPV) is a member of the feline parvovirus subgroup. It is closely related to *Feline panleukopenia virus* and *mink enteritis virus*.
CPV causes two forms of disease: Myocarditis and enteritis.
The Anigen Rapid CPV Ag Test Kit is a chromatographic immunoassay for the qualitative detection of Canine Parvovirus antigen in canine feces.
The Anigen Rapid Canine Parvovirus Ag Test Kit control line on the surface of the device. Both the test line and control line in result window are not visible before applying any samples. The control line is used for procedural control. Control line should be always appearing if the test procedure is performed properly and the test reagents of control line are working. A purple test line will be visible in the result window if there is enough Parvovirus antigen in the specimen.
The specially selected Canine Parvovirus antibodies are used in test band as both capture and detector materials. These enable the Anigen Rapid CPV Ag Test Kit to identify Canine Parvovirus antigen in canine feces with a high degree of accuracy.

2. TEST PRINCIPLES

The bulbous ends of the plastic wands have been coated with antibody to CPV. A second antibody directed against a specific CPV antigen is conjugated to the enzyme horseradish peroxidase (HRP). The fecal sample is incubated simultaneously with both the antibody-coated wand and enzyme-labeled antibodies. If antigen is present, it is captured by the wand. The enzyme-labeled antibodies are in turn captured by the antigen bound to the wand. The unbound enzyme-labeled antibody and feces are removed during the wash step and the wand is placed into a chromogenic substrate.
The development of a distinct blue color in the solution indicates the presence of CPV antigen. In the absence of CPV, no color will develop.

3. APPLICATION

The test is used to diagnose CPV infection in dogs.

4. OBJECTIVE

To describe the procedure for detection of CPV antigen

5. APPARATUS/TEST KIT/REAGENTS

5.1 Anti-CPV Antibody Coated Wands

5.2 Pre-dispensed HRP-Monoclonal Antibody Conjugate (A Tubes)
5.3 Pre-dispensed Substrate Buffer (B Tubes)
5.4 Bottle C-Chromogen (White Cap)

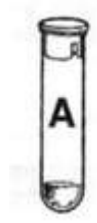
Materials required, but not provided: Marking Pen, Timer, Distilled or de-ionized water, Wash Bottle

6. PROCEDURE

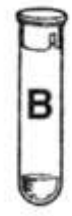
6.1 Allow kit to come to room temperature (21°-25° C; 70°-78° F) prior to use; approximately one hour.
6.2 Do not expose kit to direct sunlight.
6.3 Do not use expired reagents or mix from different kit serials.
6.4 Hold reagent vial vertically for proper drop volume.
6.5 Vaccination with modified live CPV vaccines may cause shedding of viral particles in the feces 4-10 days post-vaccination. This can cause a weak positive result.
6.6 Dispose of potentially infected specimens appropriately.

Stepwise procedure has been outlined below:
NOTE: Use canine fecal samples.
Prior to use, allow kit components to come to room temperature (70° to 78°F; 21° to 25°C).
For each sample you will need:
Anti-CPV Antibody Coated Wand. Label with dog's I

Pre-dispensed HRP-Monoclonal Antibody Conjugate Tube A.



Pre-dispensed Substrate Buffer Tube B. Label wit



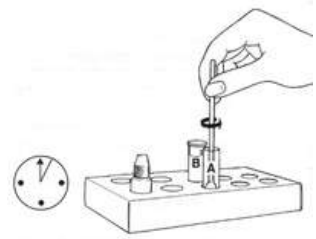
Work station with Reagent C.

Squirt Bottle with Distilled or De-ionized Water.

A. SAMPLE COLLECTION

1. Pre-wet bulbous end of Wand with de-ionized or distilled water for 3-5 seconds.

1. Swirl Wand in fecal material 3-5 seconds to cover bulbous end with a thin coat of feces.
NOTE: A specimen may be obtained rectally by inserting bulbous end of wand into rectum and *GENTLY* swirling 3-5 seconds. Do not use a lubricant.
B. CONJUGATE INCUBATION
2. Place bulbous end of Wand in tube A.
Twirl Wand vigorously until fecal material is suspended in the liquid.
WAIT 5 MINUTES.



C. PREPARE B TUBE
3. During waiting period: Remove stopper from Tube B.
Add 3 drops Bottle C (White Cap) to Tube B.
Tap to mix.
Set tube aside for use in Step 5.

D. WASH STEP

4. Remove wand from Tube A.

Wash bulbous end and tip of wand by swirling/swishing **vigorously** in a cup containing at least 250 mL of deionized or distilled water for a **minimum of 15 seconds**.

After swirling in cup, continue to wash wand by directing a **forceful stream** of deionized or distilled water against bulbous end and tip of wand and work up handle. Wash until all fecal material is removed from the wand.

Shake off excess water.

Repeat washing with a forceful stream **5-7 times.** This will ensure removal of unbound proteins which may contribute to non-specific color development.

Replace liquid in cup between wands.

NOTE: **Bloody samples may require saline rinse.**

E. COLOR DEVELOPMENT

5. Place washed Wand in Tube B.

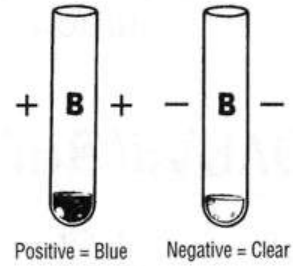
Twirl 1-3 seconds to mix.

WAIT 5 MINUTES.

Remove Wand.

7. RESULT INTERPRETATION

6. Observe solution against work-station window or a white background for blue color.



NOTE: Color intensity will vary with level of CPV present.

Optional Procedural Control

To verify technique and kit performance when a negative result is obtained:
Place wand back into Tube A.
Twirl to mix for 1-3 seconds.
Remove Wand.
Do not wash.
Place back into Tube B.

Blue color will develop within 1 minute indicating reagents were added correctly and kit is performing properly. If color does not develop, repeat the test. (This is a procedure and reagent check only. CPV antigen is not present.)

8. WASTE DISPOSAL

Dispose in proper disinfectant.

9. RISK ASSESSMENT

All specimens should be handled as being potentially infectious.

10. TROUBLESHOOTING

NA

11. REFERENCES

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Studdart, M.J. et al: Aspects of the diagnosis, pathogenesis and epidemiology of canine parvovirus. *Aust. Vet. J.* 60:197-200; 1983.

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TITLE: Rapid test for Equine Influenza

PREPARED BY: Serology & Virology section

REVISED BY: Serology & Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION
Influenza Type A viruses can infect equine species. Subtypes known to infect equine species include H3N8 and H7N7.
The FluDETECT® Equine test kit is a Rapid Immu
in the qualitative detection of Type A Equine Influenza Virus in nasopharyngeal samples from symptomatic horses.

2. TEST PRINCIPLES
The test is based on the Rapid Immuno Migration (RIM) technology. The test strip uses two antibodies that are specific to the p56 nucleoprotein of influenza virus type A. Anti-influenza A antibody bound to influenza A antigen present in the sample in the form of complex, which migrates along a strip and is captured on a sensitized reaction line by second antibody. The accumulation of the complex causes the formation of a clearly visible pink/purple band. The presence of a control band located above the reaction line, ensures that the test was performed correctly.

3. APPLICATION
This test is an in vitro designed to aid in the qualitative detection of Equine Influenza virus in nasopharyngeal samples from symptomatic horses. This assay detects all sub types of Equine Influenza virus type A.

4. OBJECTIVE
To describe the procedure for detection of Equine Influenza virus in nasopharyngeal samples.

5. APPARATUS/TEST KIT/REAGENTS
- 5.1 Desiccated vial containing 20 test strips
- 5.2 1 dropper bottle containing extraction buffer (6.0ml)
- 5.3 1 pack of 20 swabs
- 5.4 1 pack of 20 test tube
- 5.5 1 pack of 20 test tube caps
- 5.6 1 test tube rack (5 tubes)
- 5.7 Instruction for use

6. PROCEDURE

General Precautions

6.1 Do not use kit components after expiration date
6.2 Allow samples and kit to come to room temperature (+15ºC to 30ºC) before testing.
6.3 The vial holding the test strips contains a desiccant and should be kept tightly closed when not in use.
6.4 Use the test strips within 10 minutes of removing from desiccant vial.
6.5 The test strips should only be handled in the upper, labelled region, avoid contact with the surface of the test strip.
6.6 The test strip should be placed in the test tube vertically.
6.7 Use a separate swab for each sample, swabs with wooden handles or containing calcium alginate may interfere with the test and must not be used.
6.8 Do not centrifuge samples prior to use.
6.9 Do not mix materials from different test kits.
6.10 Consider all specimens potentially infectious and dispose of accordingly.
6.11 For veterinary use only.
6.12 DO NOT use swabs containing Visible Blood to limit false Positive Result.

Sample Extraction

Synbiotics Europe recommends two methods of sample extraction. If the sample will be tested using this test kit only, Follow METHOD A. If further testing is to be performed on the sample, follow METHOD B.

A

Extraction Buffer- Provided in kit box

Place 8 drops (approximately 0.25mL) of extraction Buffer in the test tube provided.

Place the swab containing the specimen in the tube and rotate the swab 5-10 times in the buffer.

When removing the swab from the tube, press the swab against the side of the tube repeatedly until no more liquid comes from the swab.

Discard the swab in an appropriate biohazard container.

If the extracted samples will not be tested immediately, cap the tube with the provided cap and store the sample according to VIII. STORAGE.

B

Brain-Heart infusion (BHI) Broth, (or alternate viral transport medium listed in Section IV) –not provided.

Place approximately 0.5mL of BHI Broth into collection tube.

Place the swab containing the specimen in the tube and rotate the swab 5-10times in the broth.

When removing the swab from the tube, press the swab against the side of the tube repeatedly until no more liquid comes from the swab.

Discard the swab in an appropriate biohazard container or break the handle of the swab below the top of the tube such that the tube containing the swab tip can be sealed with the provided cap.

If the extracted samples will not be tested immediately, cap the tube with the provided cap and store the sample according to VIII. STORAGE.

Storage

If samples will not be tested immediately they should be store at 4ºC (ice chest, cooler or refrigerator) for up to 48 hours. For prolonged storage, sample should be kept frozen (-70ºC). Do not store samples at -20ºC. Do not store samples in a self-defrosting freezer. Avoid multiple freeze-thaw cycles.

The kits should be stored between +2ºC and 30 º C. Do not freeze test kits.

Procedure

1. Testing samples –Use either method A or B as appropriate

A. Extracted in Buffer:

Remove a test strip from desiccant vial for each sample to be tested. Handle the test strip on the labelled portion of the strip.

Place the test strip directly into the test tube containing the sample. Place test strip so that the pink pad is submerged in the extracted sample. Incubate the test strip in the sample for 15 minutes.

Remove the test strip from the test tube to read.

B. Extracted in Viral Transport media:

Place 0.2 mL of the Viral Transport Media into the test tube provided.

Add 3 drops of extraction buffer to tube; tap side of tube to mix.

Remove a test strip directly into the test tube containing the sample to be tested. Handle the test strip on the labelled portion of the strip.

Place the test strip directly into the test tube containing the sample. Place test strip so that the pink pad is submerged in the extracted sample. Incubate the test strip in the sample for 15 minutes.

Remove the test strip from the test tube to read.

2. Reading Results

After 15 minutes, observe the presence or absence of pink/purple bands in the canter of the test strip between the two absorption pads.

The control band appears in the upper end of the strip (closest to the handle), while the sample test results are read in the lower part of the test strip.

Discard the test strip in an appropriate biohazard container.

7. RESULT INTERPRETATION

7.1 The test is VALID if the control line (pink/purple band) develops in the upper part of the strip. The absence of the CONTROL line indicates that the test is invalid and must be repeated.

7.2 POSITIVE for Equine Influenza Virus: Two Pink/Purple bands (Control Line and Test Line) are clearly visible on the test strip (C&T). A POSITIVE result indicates that detectable levels of influenza Virus Type A are present in the sample. Positive samples can be submitted to an approved reference laboratory for confirmation and subtype determination.

7.3 NEGATIVE for equine Influenza Virus: A single pink/purple band (Control Line) is present in the upper part of the test strip (C). A NEGATIVE result indicates that no detectable Influenza Virus Type A is presence in the sample.

7.4 Very faint lines may be due to non-specific binding and should be further investigated.

8. WASTE DISPOSAL

Dispose as per the protocol

9. RISK ASSESSMENT

NA

10. TROUBLESHOOTING

The control line on the upper part of the test strip may appear earlier. This does not mean that the test is complete. The test strip must incubate for a full 15 minutes before a sample is interpreted as negative. The test can be considered to be completed if the test line on the lower part of the stick appears before 15 minute incubation period is over. This sample is interpreted as Positive. If the test strip remains in the tube for more than 20 minutes, a false positive ghost band could appear in the place of the reaction band (T).

11. REFERENCES

Flu DETECT Equine, Zoetis Manual

TITLE: FAT for Rabies

PREPARED BY: Virology section

REVISED BY: Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION

Rabies is a fatal viral zoonotic disease of significant public and veterinary health threat. The causative agent of the disease, rabies virus (RABV), mainly infects warm-blooded vertebrates (both mammals and humans). The RABV infection is generally transmitted by the bite of infected animals, most commonly dogs and other wildlife carnivore species.

Rabies and rabies-related viruses are highly neurotropic members of the *Lyssavirus* genus, family *Rhabdoviridae*. Within this family of viruses are seven genotypes (gts) (1-7), namely:- classical rabies virus (gt1), Lagos bat virus (LBV, gt2), Mokola virus (MOKV, gt3), Duvenhage virus (DUVV, gt4), European bat lyssavirus type-1 and 2 (gts 5 & 6 respectively) and the Australian bat lyssavirus (ABLV, gt7) as well as unassigned viruses.

Virus factories in infected cells appear as inclusion bodies, consisting predominantly of viral capsids (Ribo-Nucleo-Protein, RNP). The histologically demonstrable rabies inclusion bodies are called "Negri bodies ". Among the laboratory tests available for routine diagnosis, the fluorescent antibody test (FAT), which detects viral antigen is the gold standard and should be used by all laboratories performing such work. The test is fast (results are obtained in less than 3 hours), comparatively inexpensive and more accurate (can detect 97-99% of positive specimens) than either histological based or mouse inoculation tests. FAT is the recommended by both the WHO and the OIE. Negative samples with human contact (bite) history should be subjected to viral propagation techniques (mice or cell cultures) and subsequently confirmed by FAT.

2. PRINCIPLES

The smears are incubated with FITC-labelled anti-lyssavirus polyclonal antibody. Un-bound antibody is then removed by washing and smears are examined by fluorescence microscopy. In rabies positive specimens the antibodies bind to the antigen and produce apple-green fluorescing inclusion bodies or viral aggregates when viewed under a fluorescent microscope.

3. APPLICATION

The test is used for diagnosing Rabies in animals.

4. OBJECTIVE

To describe the FAT method in detecting rabies inclusion bodies (viral antigen) in cells of acetone-fixed brain smears.

5. APPARATUS/TEST KIT

5.1 Fluorescent microscope, Zeiss or equivalent, with mercury vapour lamp, 50 or 100 watt (450- 490 nm excitation filters, and 510 nm stop filter).
5.2 Refrigerator (+2 to +8°C).
5.3 Freezers, chest or upright, with temperature of not less than -20°C (e.g. -70^{O}C).
5.4 Incubator, temperature capability of 37°C±2°C).
5.5 Double door autoclave, any brand.
5.6 Class II biological safety cabinets, any approved brand.
5.7 Single channel Micropipettor for drawing 100µl volumes.
5.8 Single channel Micropipettor for drawing 0.5-10µl volumes.
5.9 Vortex mixer.
5.10 pH meter.
5.11 Dark room.
5.12 Forceps.
5.13 Scissors.
5.14 Standard laboratory timers.
5.15 Closed plastic container for use as a humidified chamber
5.16 Microscope slides, clear, frosted one end, any brand, non-fluorescent
5.17 Cover slips 13 mm thick, any bran.
5.18 Petri dishes, disposable or other suitable container to place brain material
5.19 Non fluffing high quality absorbent paper towel.
5.20 Coplin staining jar
5.21 HB pencil for labelling slides.
5.22 Lens cleaning tissue
5.23 Squeeze/wash bottle with PBS
5.24 A suitable containers with virucidal disinfectant capable of killing rabies virus
5.25 PPE including Nitrile/Latex powder free disposable Gloves
5.26 Disposable tips (1-10 µl, 20-200 µl,)
5.27 Autoclavable bags

6. REAGENTS, SOLUTION AND BUFFER

6.1 0.01M Phosphate buffered saline (PBS), pH 7.2-7.4
6.2 Heat sterilized distilled or deionized water, or water of an equivalent purity.
6.3 High-grade (99.9 to 100%) acetone (CH3)2CO (MW 58.8), Assay by (GC) is minimum of 99.5%, ASC grade, or similar.
6.4 High grade glycerol
6.5 Anti-lyssavirus FITC polyclonal conjugate (can be obtained from the ARC-Onderstepoort Veterinary Institute (www.arc.agric.za), SANOFI Pasteur (www.sanofipasteur.com), Chemicon (www.chemicon.com) with a predetermined working dilution.
6.6 Lens cleaning fluid.
6.7 Mounting media/mountant.
6.8 Positive and negative control brain specimens.

6.9 Fresh or 50% glycerol saline preserved test brain specimen.

6.10 Evans blue. Counterstain added to the working dilution of the conjugate is optional. Evans Blue counterstain (0.5%) can be aliquoted and stored at +4°C for up to 6 months and indefinitely at -20°C. The amount of counterstain added to the conjugate is determined by titration when the working dilution of the conjugate is determined. Due to counterstain, the cells will be noticeably red, but should not be strongly red as to diminish the specific green fluorescence. An Evans Blue concentration of 0.00125% works very well in many laboratories. This concentration is prepared by adding 2.5 µl of 0.5% stock dye solution per ml of conjugate diluent.

7. PROCEDURE

7.1 Prepare the diluents for the conjugate which is 0.01M PBS pH 7.2±0.2 with 0.5% Evans blue. This can be prepared and aliquoted into 1 ml volumes at -20°C.

7.2 Determine the optimum working dilution of the anti-lyssavirus FITC polyclonal conjugate as per manufacturer instructions to provide an excellent staining of viral antigen in brain smears. On the day of specimen testing, prepare only enough of the optimum working dilution conjugate for the number of samples to be tested by adding working dilution of the conjugate into the diluent above (PBS with 0.5% Evans Blue).

7.3 On each day of performing a test, ensure that there is sufficiently chilled acetone preferably kept -20oC.

7.4 Keep microscope slides in an acetone bath at room temperature for degreasing and take them out when needed for staining.

7.5 Dry the slides using appropriate absorbent paper towel.

7.6 Phosphate buffered saline (PBS) 0.01M pH 7.2-7.4 (Refer to Appendix 2 for instructions on how to prepare the buffer).

7.7 Keep fresh filtered acetone in a Coplin or staining jar in a freezer (-20°C).

7.8 Preserve fresh brain specimens that cannot be tested on the day of arrival in 50% glycerol- saline (refer to Appendix 3 for instructions to prepare glycerol-saline). Specimens in 50% glycerol-saline can be stored at room temperature for a maximum of 2 weeks, or frozen at -20°C or at a lower temperature overnight.

7.9 Thaw frozen control samples on the day of testing by placing them in a bio-safety cabinet at room temperature for 30-60 minutes.

7.10 Prepare mounting media (Refer to Appendix 4 for instructions on how to prepare the mounting media).

8. RESULT INTERPRETATION

8.1 Two people must read all the stained slides and control smears. Positive controls must be positive and the negative control sample must be negative for the results to be acceptable.

8.2 The stained slides should be examined using the 40X objective starting with positive and negative controls. In positive controls, the presence of rabies antigen is demonstrated by appearance of apple green oval to round shaped inclusion bodies of various sizes, some as dust particles studded against a

black to dark greenish background, while such fluorescing inclusion bodies should not appear in negative control smears.

8.3 Continue reading the test smears.

8.4 Grade the smear quantitatively with a score of +1 to +4, depending on the abundance of viral antigens and give a quantitative grade of +1 to +4 depending on the intensity of fluorescence and record the results in the appropriate test results recording system as Positive, Negative or Doubtful

9. WASTE DISPOSAL

Since the virus can infect the humans also, appropriate method needs to be followed for disposal of the specimens, slides and other equipment.

10. RISK ASSESSMENT

10.1 All laboratory personnel who handle and work with suspected rabies virus infected tissue specimens must be well trained, competent and comply with national biocontainment and biosafety regulations to protect staff from contact with pathogens.

10.2 All personnel involved in rabies testing should receive pre-exposure immunisation.

10.3 Only personnel who demonstrate an antibody titre of 0.5 international units (IU) per ml or higher should be allowed to handle the suspected rabies infected specimens.

10.4 Personnel should be routinely monitored every 6 months for adequate rabies neutralising antibodies. Booster vaccinations must be given when the titre falls below 0.5 IU/ml. In the absence of serological monitoring, the vaccination regimen should consist of a booster vaccination at 1 year and thereafter every 1-3 years, depending on the vaccine.

10.5 Appropriate protective clothing must be worn at all times.

10.6 The specimens must only be processed in a Class II Biological Safety Cabinet.

10.7 Aerosols – high speed centrifugation and any procedure that generates aerosols should be carried out in tightly closed containers and possibly under a negative draught hood.

10.8 All contaminated instruments and utensils must be sterilised by autoclaving immediately after the test procedure is finished and before they are washed.

10.9 Disposable items must be placed into sterilising bags and must be sterilised by autoclaving before disposal.

10.10 Disinfection of biological safety cabinets and used instruments should be done with disinfectants such as 1% Virkon and/or other virucidals efficient to kill lyssaviruses.

11. TROUBLESHOOTING

11.1 Each new lot of acetone must be checked to ensure that the acetone does not interfere with the staining of the brain smears.

11.2 Select tissue from 4 to 6 previous specimens that have stained both weakly and strongly positive for rabies virus.

11.3 Prepare two slides from each specimen.
11.4 Fix one set of slides in the acetone in use and the other set in the new lot of acetone.
11.5 Follow the procedures for fixing and staining.
11.6 Read both sets of slides noting the amount of virus as well as intensity of staining.
11.7 Both sets of slides must have identical results. If the new acetone does not meet this criterion, discard and purchase another lot.
11.8 Similarly, the optimal working dilution of a new batch of conjugate must be determined by titration. Naturally, the working dilution may differ between laboratories depending on the microscope optimal system.
11.9 Select known positive and negative brain material to prepare controls.
11.10 Inoculate suckling mice with a 10% suspension of sample that previously tested positive with a score of +4. Pool brain samples from which succumb and test positive for rabies virus antigen. To prepare the negative control, inoculate suckling mice with PBS. Harvest brain tissues after 28 days, pool and place in vials.
11.11 Prepare two-fold dilutions of the conjugate in PBS starting from 1:80 to 1:2560.
11.12 Prepare two slides for each specimen.
11.13 Fix the slides as described in the protocol.
11.14 Follow the procedures for fixing and staining.
11.15 Read both sets of slides noting the amount of virus and intensity.
11.16 The least conjugate dilution that gives an excellent staining will be used as a working dilution of that batch.
11.17 Prevent conjugate contamination by dispensing 1-ml quantities of the diluted conjugate into eppendorf tubes and store at -20^{O}C or lower.
11.18 External quality control will involve an annual participation by our laboratories in an FAT proficiency testing on a panel of rabies positive and negative samples

12. REFERENCES

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Vaccines for Terrestrial Animals. Fifth Edition. OIE. Paris de Prony.

Melsin, F.X., Kaplan, M.M and Koprowski, H. 1996. Laboratory techniques in rabies, fourth edition. WHO, Geneva.

TITLE: Rabies immunoperoxidase antigen detection (RIAD)

PREPARED BY: Virology section

REVISED BY: Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION

Rabies is a fatal viral zoonotic disease of significant public and veterinary health threat. The causative agent of the disease, rabies virus (RABV), mainly infects warm-blooded vertebrates (both mammals and humans). The RABV infection is generally transmitted by the bite of infected animals, most commonly dogs and other wildlife carnivore species.

Rabies and rabies-related viruses are highly neurotropic members of the *Lyssavirus* genus, family *Rhabdoviridae*. Within this family of viruses are seven genotypes (gts) (1-7), namely:- classical rabies virus (gt1), Lagos bat virus (LBV, gt2), Mokola virus (MOKV, gt3), Duvenhage virus (DUVV, gt4), European bat lyssavirus type-1 and 2 (gts 5 & 6 respectively) and the Australian bat lyssavirus (ABLV, gt7) as well as unassigned viruses.

Virus factories in infected cells appear as inclusion bodies, consisting predominantly of viral capsids (Ribo-Nucleo-Protein, RNP). The histologically demonstrable rabies inclusion bodies are called "Negri bodies ". Among the laboratory tests available for routine diagnosis, the fluorescent antibody test (FAT), which detects viral antigen is the gold standard and should be used by all laboratories performing such work. The test is fast (results are obtained in less than 3 hours), comparatively inexpensive and more accurate (can detect 97-99% of positive specimens) than either histological based or mouse inoculation tests. FAT is the recommended by both the WHO and the OIE. Negative samples with human contact (bite) history should be subjected to viral propagation techniques (mice or cell cultures) and subsequently confirmed by FAT.

2. PRINCIPLES

It is indirect Immunoperoxidase detection of Rabies antigen in brain smears. It uses an in house produced anti-Rabies rabbit serum against an expressed Rabies virus nuclear protein in *E.coli*. The secondary antibody is an anti-rabbit peroxidase conjugate and uses AEC as the substrate/chromogen for detection. This enables stained Rabies antigen to be read with a light microscope.

3. APPLICATION

The test is used for rapid diagnosis of Rabies in animals.

4. OBJECTIVE

To describe the method of RIAD for diagnosis of rabies in animals.

5. APPARATUS/TEST KIT

5.1 Class II Biosafety Cabinet
5.2 Fumehood
5.3 Light microscope
5.4 Humidified chamber (plastic)
5.5 Petri dish, 90mm
5.6 Scalpel with handle (disposable no: 22)
5.7 Glass slides or positively charged slides-commercial
5.8 Coverslips 50mm x 22mm
5.9 Glass slides plain
5.10 Slide rack-metal or glass not plastic
5.11 Polypropylene and glass Coplin slide jar
5.12 10ml sterile polypropelene tubes
5.13 Sharps and Biological bin
5.14 Pipettes –currently verified for use
5.15 Sterile plugged 200µl & 1ml tips
5.16 Sterile dissecting instruments
5.17 Autoclave
5.18 –80°C Freezer (for reagent storage),
5.19 4°CRefrigerator (for reagent storage)
5.20 -20°C freezer (fixing)

6. REAGENTS, SOLUTION AND BUFFER

Chemicals

6.1 Silane(AAS) 3-aminopropyltriethoxysilane(Mw. 221.37) Sigma cat# A36-48
6.2 100% Acetone (Analar)
6.3 100% Ethanol(Tech Grade)
6.4 Tris Buffer pH7.6 DAKO cat # K8007 (or in house recipe see appendix)
6.5 30% Hydrogen Peroxide H_2O_2
6.6 AEC 3-Amino-9-ethyl-carbazole (sigma product) Sigma cat# A5754
6.7 N,N,Dimethylformamide (DMF) Sigma cat# D4551-250
6.8 Sodium Acetate or Sodium Acetate anhydrous
6.9 Glacial Acetic Acid
6.10 Distilled water pH=6.9-7.2
6.11 Virkon or equivalent disinfectant
6.12 Skim milk powder
6.13 Lilly Meyer's Haemotoxylin counter stain

Biologicals

6.14 Rabbit anti Rabies polyclonal serum #663(AAHL reagent)

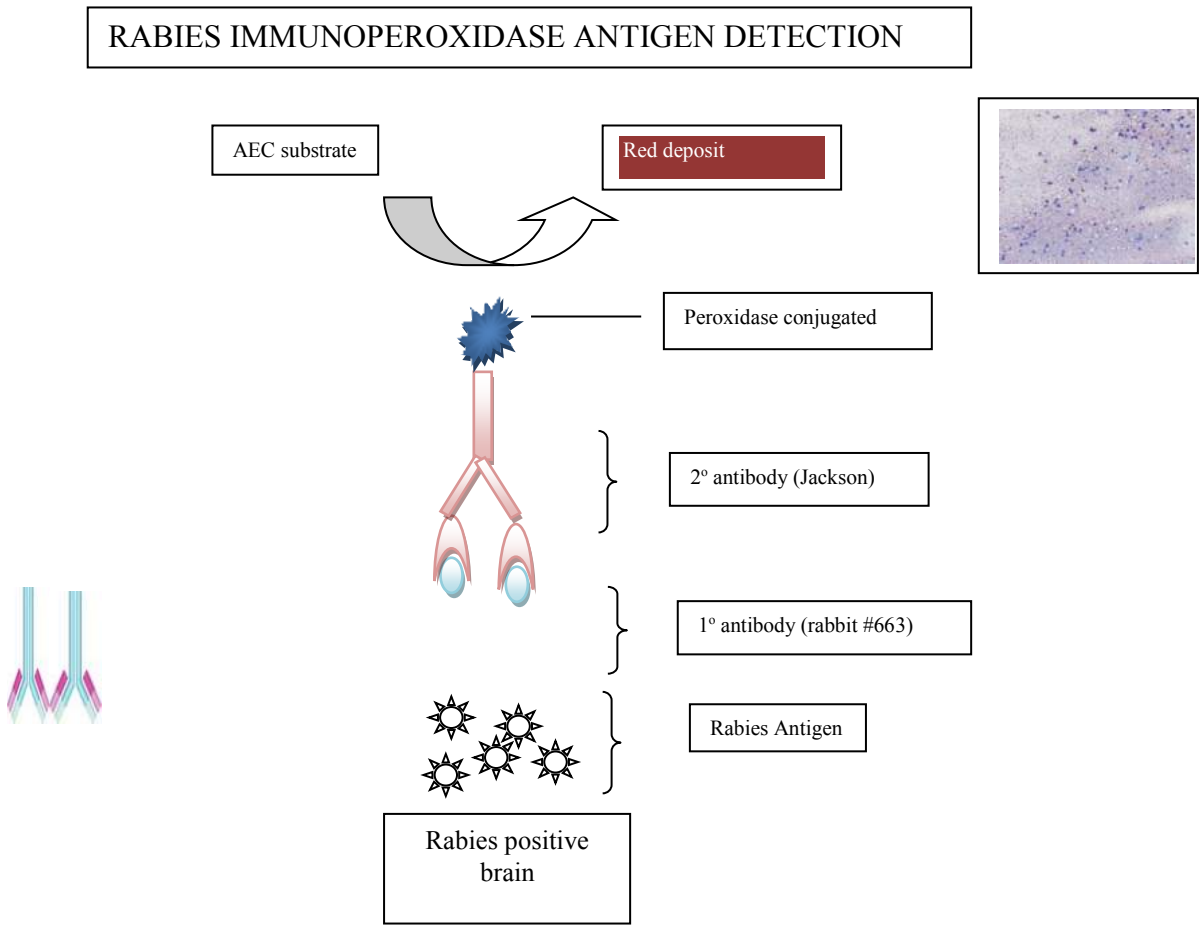
6.15 Anti-Rabbit HRPO conjugated antibody Jackson cat# 711-036-152

6.16 Positive control Rabies infected brain

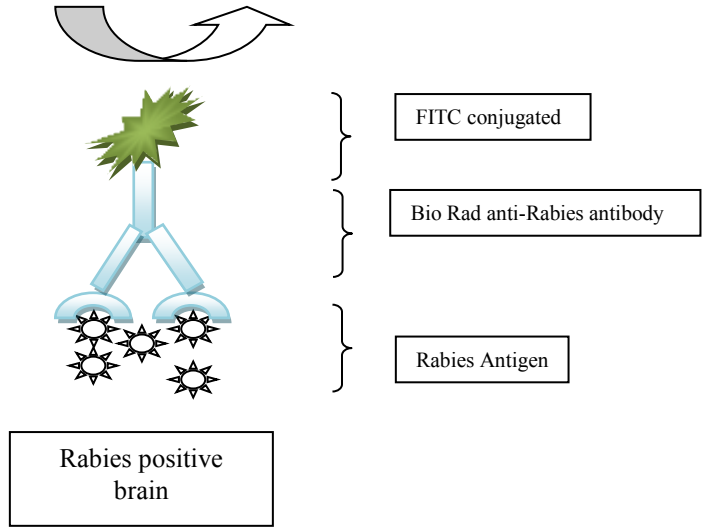
6.17 Negative brain (not Rabies infected)

7. PROCEDURE

Schematic Representation of Rabies FAT and RIAD tests



RABIES FLUORESCENT ANTIBODY TEST (FAT)



Preparation of Brain Smear –Slip Smear Technique

This method is preferred to the impression smear technique as it gives a thin film smear, which reduces non-specific binding of the antibodies during the detection steps. Working inside a Class II Biosafety Cabinet

Place the brain in a 90mm Petri dish (larger brains can be worked with on a large clean plastic bag).
Identify brain stem, cerebellum, hippocampus and cerebral cortex. (See CDC Rabies DFA technique for descriptive images).
Remove 0.5cm^{3}portionsof each of the nominated regions to a clean Petri dish, cutting with a sterile handled scalpel blade.
If it is not possible to identify these regions of the brain then remove tissue from four separate sections of the tissue.
Cut the tissue with the scalpel blade finely and place a small amount on the surface of an AAS treated slide (see Appendix 7.1). Place a second slide underneath the test slide for support.
Place the two slides along your index finger of your left hand for extra support.
Produce the smear by using a 100% Ethanol cleaned slide held in your right hand, placing it on top of the tissue with your thumb over the top slide, pressing down and sliding the top slide away from the bottom 2 slides.
Allow the brain smear to dry.
Discard the top slide, the supporting bottom slide can be re-used to make the other smears.

Slide Fixation

1. Place air dried smears into a fresh bath of cold -20ºC 100% Acetone (make sure the container has a lid).
2. Fix for 30-45minat -20ºC.
3. Remove from the Acetone and allow to dry in the Class II Biosafety cabinet

Rabies Antigen Detection-Immunoperoxidase

General notes before starting

See Appendix for buffer recipes

It is very important that the distilled water has a pH 6.9-7.1 (please check before commencing)
All incubations are done at room temperature 21-25ºC
Rinse well after each step for 2 min.
Have all the reagents at 21-25ºC temperature for use.
Keep the slides moist at all times-very important (if slides dry out non-specific staining will result).
Have the reagents for the next step prepared before the wash.
Prepare a squeeze bottle with Tris Buffer and one with distilled water for rinses.
Prepare fresh Tris Buffer with 1% skim milk
Dilute the Antibodies in Tris Buffer with 1% Skim Milk Powder.
The test is done in a humidified container to prevent the smears from drying
- -moisten some lint free tissue with distilled water and place in a plastic container with a lid.

Include a positive and negative control with each test

Antigen detection

6.1 Prepare the 3% Hydrogen Peroxide blocking solution
6.2 Prepare the Antibody Diluent(1% Skim Milk in 1X Tris buffer pH7.6)

3% Hydrogen Peroxide	Volume
30% Hydrogen Peroxide stock	1ml
Distilled water	9ml

6.3 Rinse the smears through 3 serial baths of Tris-Buffer to moisten the smears

1X DAKO Tris pH7.6	Volume
20X Tris pH7.6 stock	50ml
Distilled water pH=7.0	950ml

6.4 Place smears in the humidified container at Room Temperature

6.5 Treat the each slide with 300µl of 3% Hydrogen Peroxide,10min at room temperature (close the lid- to keep moist)
6.6 Dilute the Rabies polyclonal serum (Rabbit#662) 1:1000in antibody diluent

1o Antibody (Rabbit #663) 1:1000	Volume
Rabbit #663 antibody	2 µl
Antibody Diluent	2000µl

6.7 Rinse the slides through 3 serial baths of Tris-Buffer (the last bath should have fresh Tris buffer)
6.8 Place back into the humidified container at room temperature add 300µl of the 1o antibody diluted 1:1000 per smear
6.9 Incubate at room temperature 45minin the humidified chamber
6.10 Dilute the 2o Antibody(Jackson)1:500in antibody diluent
6.11 Rinse the slides through 3 serial baths of Tris-Buffer as in step 7
6.12 Add 300µl of the 2o antibody diluted at 1:500 per smear

2º Antibody (Jackson) 1:500	Volume
2º Antibody	4 µl
Antibody Diluent	2000µl

6.13 Rinse the slides through 3 serial baths of Tris-Buffer as in step 7
6.14 Add 300µl of the 2o antibody diluted at 1:500 per smear

AEC Substrate	Amount
AEC powder(pre-weighed)	2mg
Di-Methyl-Formamide (DMF)	500µl
Mix well	
0.05M Acetate buffer pH5.0	9500µl
30% $H_20_{2(}$ **just before use)**	5µl

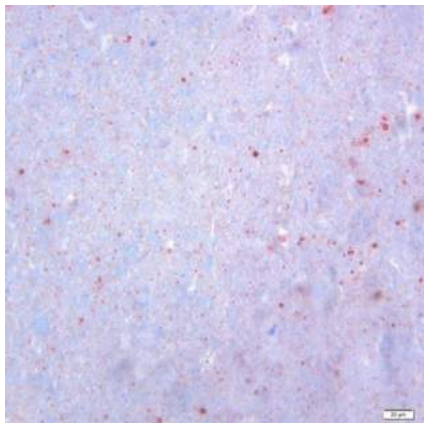
6.15 Wash slides 3 times with Tris Buffer
6.16 Activate the AEC substrate with 5µl 30%Hydrogen Peroxide (H2O2)
6.17 Add 300 µl of activated substrate for each brain smear
6.18 Incubate at room temperature for 10min-monitor colour development
6.19 Wash with distilled water pH7.0 once (to stop the reaction)
6.20 Counter Stain with Lilly Meyers Haemotoxylin 20 sec
6.21 Rinse excess stain off with distilled water and wash 1X in distilled water bath
6.22 Dip slides into Tris buffer
6.23 Rinse and leave in distilled water until cover slipping
6.24 Coverslip using aqueous mounting medium

8. RESULT INTERPRETATION

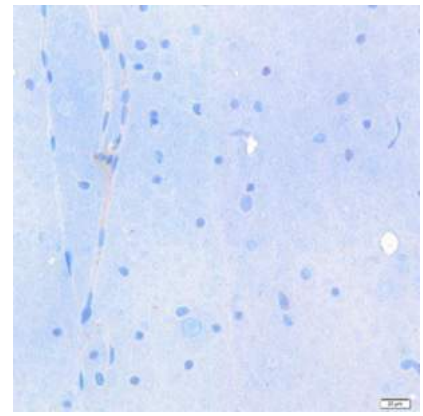
Slides are read under a light microscope- 40X lens

The presence of Rabies antigen is seen as brick red deposits in the smear against blue brain tissue. It is important to distinguish non-specific staining especially on the edges of the smear. For this reason positive and negative control smears must always be included with each test.

Smears are scored as positive or negative for antigen. A description of the quantity of antigen present can be included, for example high or low positive.



Positive Canine brain



Negative Canine smear

9. WASTE DISPOSAL

Since the virus can infect the humans also, appropriate method needs to be followed for disposal of the specimens, slides and other equipment.

10. RISK ASSESSMENT

10.1 All laboratory personnel who handle and work with suspected rabies virus infected tissue specimens must be well trained, competent and comply with national biocontainment and biosafety regulations to protect staff from contact with pathogens.

10.2 All personnel involved in rabies testing should receive pre-exposure immunisation.

10.3 Only personnel who demonstrate an antibody titre of 0.5 international units (IU) per ml or higher should be allowed to handle the suspected rabies infected specimens.

10.4 Personnel should be routinely monitored every 6 months for adequate rabies neutralising antibodies. Booster vaccinations must be given when the titre falls below 0.5 IU/ml. In the absence of serological monitoring, the vaccination regimen should consist of a booster vaccination at 1 year and thereafter every 1-3 years, depending on the vaccine.

10.5 Appropriate protective clothing must be worn at all times.

10.6 The specimens must only be processed in a Class II Biological Safety Cabinet.

10.7 Aerosols –high speed centrifugation and any procedure that generates aerosols should be carried out in tightly closed containers and possibly under a negative draught hood.

10.8 All contaminated instruments and utensils must be sterilised by autoclaving immediately after the test procedure is finished and before they are washed.

10.9 Disposable items must be placed into sterilising bags and must be sterilised by autoclaving before disposal.

10.10 Disinfection of biological safety cabinets and used instruments should be done with disinfectants such as 1% Virkon and/or other virucidals efficient to kill *Lyssa viruses*.

11. TROUBLESHOOTING

NA

12. REFERENCES

Rahmadane I, Certoma AF, Peck GR, Fitria Y, Payne J, Colling A, et al. (2017) Development and validation of an immunoperoxidase antigen detection test for improved diagnosis of rabies in Indonesia. PLoS Negl Trop Dis 11(11): e0006079. https://doi.org/10.1371/journal.pntd.0006079

13. APPENDIX

13.1 Silane treated slides preparation

This pre-treatment can help the tissue to stick to the slide if this is a problem.
Wear gloves, laboratory coat and safety glasses.
Work in a fume hood with good ventilation.
Prepare 3X 100% Ethanol baths/pots for rinsing.
Reagents

3-aminopropyltriethoxysilane (MW 221.37)	2.0ml (Sigma cat# A36-48)
100% Acetone (Analar)	200ml

Prepare fresh-keep the silane solution dry this is important so that slides are not blotchy
Use metal slide racks and glass pots for rinses as the Silane sticks to plastic

1. Pre-clean glass slides by soaking them in 100% Ethanol or wash
2. Dry the slides with a kim-wipe (lint free tissue-this is important)
3. Place slides into a metal rack (not plastic) and dip into the Silane solution, gently rock to ensure even coating
   Do this 10times
4. Rinse slides through 3 serial Ethanol baths- 10 dips each bath
5. Ensure the last bath has fresh clean Ethanol
6. Dry slides in the Fume hood for 5 min then place in a dry clean oven at 37^{o}C overnight. If not available dry overnight at room temperature with a lint free tissue covering the slides.

Keep slides dry in a clean container at room temperature until needed.

13.2 Solutions

3% Hydrogen Peroxide

Dilute 30% Hydrogen Peroxide 1:10 with distilled water
Need 300µL per smear

10X Tris Buffered Saline

REAGENT	QUANTITY SUPPLIER/CAT#	
Trizma Base Trizma HCl NaCl (Analar) Tween 20 1N HCl Distilled Water	13.9g 60.6g 87.66g 5ml 20ml 1.0 L	 Sigma T-1503 Sigma T-3253

Tris Buffered Saline Working Stock
10X Tris Buffered Saline 1.0L
Distilled Water 9.0L
pH before use pH 7.6

20X DAKO Tris Buffered Saline Concentrate (DAKO cat #K8007)
20X Concentrate 500ml
Distilled water 9.5L
pH before use pH7.6

ANTIBODY DILUENT
Tris Buffer 1% Skim Milk

REAGENT	QUANTITY
1X Tris Buffer pH7.6	50ml
Skim milk powder	0.5g

Stock Acetate Buffer (0.1M pH 5.0)

REAGENT	QUANTITY
Sodium acetate OR Sodium acetate anhydrous	12.0 g 8.20g
Distilled Water	To 1000ml
Glacial Acetic Acid	(requires approx. 1.0 ml) pH to 5.0

Working stock Acetate Buffer (0.05M - pH 5.0)
Distilled Water 100ml
Acetate Buffer (0.1M pH 5.0) 100ml

AEC Chromagen Solution

AEC- 3-Amino-9-ethyl-carbazole (AEC) 4mg
Di methyl Formamide 1ml
Dissolve the AEC well

0.05 Acetate Buffer pH5.0 19ml
Just before use activate the AEC with:
30% Hydrogen Peroxide 10µl

Measure 40% Formaldehyde out in a fume cupboard

Mayer's Haemotoxylin (Lillie's modification)

Reagent	Amount
Haemotoxylin (CI# 75290)	5.0
Ammonium Alum	50.0g
Glycerol	300ml
Water	700ml
Sodium Iodate (chemical ripening agent)	0.2-0.4g
Acetic Acid	20.0ml

Procedure

Dissolve the Haemotoxylin with 350ml water with a gentle heat
Dissolve the Ammonium alum in 350ml water
Cool both solutions to 25^0C
In a 2L bottle or conical flask add the dissolved ammonium alum to the Haemotoxylin, stirring
Add the sodium iodate
Add the glycerol and acetic acid

The solution is ready to use.

Commercially: Lillie Mayer Haemotoxylin (modified) Australian Biostain cat# AHLM 5L

Neat solution ready to use

TITLE: Foot & Mouth Disease NSP AB test

PREPARED BY: Serology & Virology section

REVISED BY: Serology & Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious viral disease that affects both domesticated and wild cloven-hoofed animals, such as cattle, sheep, and pigs.

After FMDV infection, the virus will produce structural proteins (SPs) and non-structural proteins (NSPs). These proteins are both involved in further virus replication; the former form the viral particles, while the latter are specific to the replication process. As the virus replicates, NSPs will enter the blood circulation causing immune reactions and produce NSP antibodies.

Quicking Foot and Mouth Disease NSP Ab Rapid Test (**Cat No.: W81058, quicking)** is a sandwich lateral flow immunochromatographic assay for the qualitative detection of Foot and Mouth Disease Non-structural Proteins antibody (FMD NSP

2. TEST PRINCIPLES

Quicking Foot and Mouth Disease NSP Ab Rapid Test is based on sandwich lateral flow immunochromatographic assay. The test device has a testing window. The testing window has an invisible T (test) zone and C (control) zone. When sample is applied into the sample hole on the device, the liquid will laterally flow on the surface of the test strip. If there is enough FMD NSP antibodies in the sample, a visible T band will appear. The C band should always appear after a sample is applied, indicating a valid result. By this means, the device can accurately indicate the presence of FMD NSP antibodies in the sample.

3. APPLICATION

This assay is used for the qualitative detection of Foot and Mouth Disease Non-structural Proteins antibody (FMD NSP Ab) in animal's ser

4. OBJECTIVE

To describe the procedure for detection of Foot and Mouth Disease Non-structural Proteins antibody (FMD NSP Ab) in animal's serum

5. APPARATUS/TEST KIT/REAGENTS

5.1 10×foil pouches, each containing one cassette, one pipette and a desiccant
5.2 10×centrifugal tubes
5.3 Product Manual

6. PROCEDURE

6.1 Do centrifugation to animal's whole blood use. If serum is too sticky to move, please do a dilution of 1:1 with PBS or distilled water.

6.2 Take out the cassette from the foil pouch and place it horizontally.

6.3 Gradually drip 3 drops of serum/plasma in

6.4 Interpret the result in 5-10 minutes. Result after 10 minutes is considered as invalid.

7. RESULT INTERPRETATION and REPORTING

Positive: The presence of both C band and T band, no matter T band is clear or vague.
Negative: Only clear C band appears.
Invalid: No colored band appears in C zone, no matter whether T band appears.

8. WASTE DISPOSAL

8.1 Dispose the tests materials safely

8.2 Ensure that the test materials (swabs, pipettes, vials, containers etc.) are properly packed and are brought back to the laboratory for proper disinfection by autoclaving and disposed of safely in the pit.

8.3 Alternatively, the test materials and samples should be disinfected in 2% Virkon-S and autoclaved

9. RISK ASSESSMENT

9.1 Use full personal protective equipment (PPE) for suspect cases

9.2 While doing post mortem, maximum precautions are to be taken (this is a technical discipline to be followed every time).

9.3 All specimens from suspected cases should be considered potentially infectious and ensure personal safety precautions

10. TROUBLESHOOTING

10.1 Viscous sample preparation can prevent lateral flow of sample and reaction leading to delayed reading time.

10.2 Avoid bubble formation while loading sample on cassette

11. REFERENCES

Manual for Foot and Mouth Disease NSP Ab test cat no. W81058

TITLE: Bovine Brucella Ab test

PREPARED BY: Serology & Virology section

REVISED BY: Serology & Virology section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION
Quicking Bovine Brucella Ab Rapid Test (**Cat No.: W81085, quicking)** is a sandwich lateral flow immunochromatographic assay for the qualitative detection of Bovine Brucella antibody in animal's serum or plasma.

2. TEST PRINCIPLES
This test strip is based on fast immuno-chromatography technique to detect Brucella Ab in bovine serum, plasma and whole blood qualitatively. When testing, Brucella Ab in sample combine with antigen coated by colloid gold forming complexes, moving forward to the other head. When reaching the T-line, the specific antigen on the membrane capture the complexes and appear a T line, C line appears, means the test is valid. The T line appears, means there is Brucella Ab in the sample.

3. APPLICATION
This kit is used for the qualitative detection of Bovine Brucella antibody in animal's serum or plasma.

4. OBJECTIVE
To describe the procedure for qualitative detection of Bovine Brucella antibody in animal's serum or plasma.

5. APPARATUS/TEST KIT/REAGENTS
5.1 10 x foil pouches, each containing one cassette, one pipette and a desiccant
5.2 1 x buffer assay (5.0ml)
5.3 10 x pipette/dropper 1(5µl)
5.4 10 x centrifugal tubes
5.5 Product Manual

6. PROCEDURE
Whole blood

Collect anticoagulated whole blood, use for that very day;

4.2 Serum

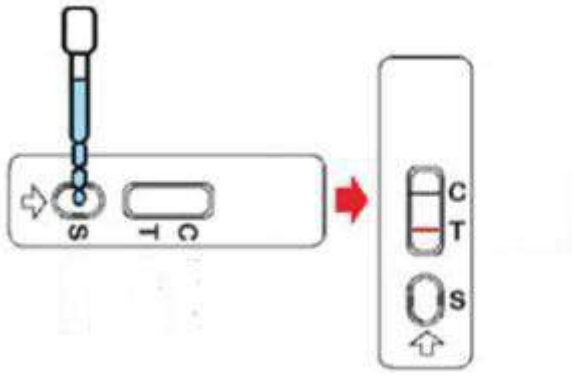
Collect blood, put at 37 for 1-2h, take the supernatant, centrifuge at 1500r/min for 10min, separate the serum. The serum can store for 2-3 days at 4 , for long term storage, store at -20 in frozen.

Operation procedures

1) Read the manual carefully and return the test card and sample to room temperature before use.

2) Take out test card, use dropper to absorb the sample, drop 1 drop of sample into buffer, mix evenly, then take 2-3 drops into well mark " S when the sample quantity is small, can use Micropipette to transfer 5ul sample into well mark " S first, then add 2-3 drops of buffer to the well.

3) Read the results in 15min at room temperature.



The test card can be used only once at room temperature, do not reuse or use test card out of expiry date.

Return all reagents and sample into room temperature before use.

Be careful when prepare sample, wear glove and mask.

The whole blood need add anticoagulants, not frozen; serum sample can store in frozen, avoid repeated freezing and thawing.

This product is only for in vitro rapid diagnostic veterinary use, cannot be quantified, do only qualitative testing.

The product test results for reference only, for confirmation, please refer to the relevant national standard methods.

Storage and expiry date

Storage: Store at 4-30 in dark, sealed, dry place, no frozen.

Expiry date: 12 months; date of production is on box.

Do centrifugation to animal's whole blood sample to col

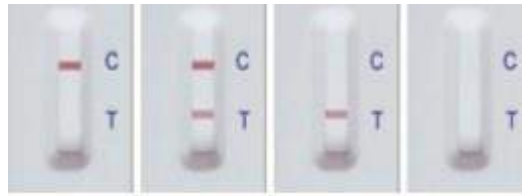
If serum is too sticky to move, please do a dilution of 1:1 with PBS or distilled water.

Take out the cassette from the foil pouch and place it horizontally.

Gradually drip 3 drops of serum/plasma into the sample hole " S

Interpret the result in 5-10 minutes. Result after 10 minutes is considered as invalid.

7. **RESULT INTERPRETATION**
8. Results



Negative Positive Invalid Invalid

- Negative**(-)**: no color reaction on Test Line (T Line),only on Control Line(C Line) being seen red color reaction. It means there is no Brucella Ab in sample or the levels is lower than the detection threshold;
  Positive**(+)**: Both on T Line and C line being seen red color reaction. It means there is Brucella Ab in sample;
  Invalid**:** No color reaction on C Line. It means the operation is wrong or the test card is invalid.

9. WASTE DISPOSAL

9.1 Dispose the tests materials safely
9.2 Ensure that the test materials (swabs, pipettes, vials, containers etc.) are properly packed and are brought back to the laboratory for proper disinfection by autoclaving and disposed of safely in the pit.
9.3 Alternatively, the test materials and samples should be disinfected in 2% Virkon-S and autoclaved

10. RISK ASSESSMENT

10.1 Use full personal protective equipment (PPE) for suspect cases
10.2 While doing post mortem, maximum precautions are to be taken (this is a technical discipline to be followed every time).
10.3 All specimens from suspected cases should be considered potentially infectious and ensure personal safety precautions

11. TROUBLESHOOTING

11.1 Viscous sample preparation can prevent lateral flow of sample and reaction leading to delayed reading time.
11.2 Avoid bubble formation while loading sample on cassette

12. REFERENCES

Manual for Bovine Brucella Ab test cat no. W81085

TITLE: Canine *Leptospira* Antibody test

PREPARED BY: Virology Section

REVISED BY: Virology Section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. INTRODUCTION

Canine leptospirosis is a potentially deadly disease caused by Leptospira serovars *Grippotyphosa, Canicola, Pomona, and Icterohaemorrhagiae.*Timely diagnosis and treatment is essential. The SNAP Lepto Test enhances helps to test for antibodies to leptospirosis and start treatment immediately.

It is in vitro diagnostic for the detection of anti-Leptospira antibodies to the serovars *Grippotyphosa, Canicola, Pomona, and Icterohaemorrhagiae* in canine serum.

2. TEST PRINCIPLES

SNAP ELISA uses reverse directional flow, a wash step to eliminate nonspecific binding, an enzymatic reaction step to amplify signal development, and a distinct blue-colored reaction product that is easy to observe against the background of the white-colored matrix.

In the SNAP ELISA, there is little or no background color, and blue-colored positive results are easily interpreted because of the high color contrast between spot and sample color.

3. APPLICATION

The test is used to detect the antibody against *Leptospira* in the serum sample.

4. OBJECTIVE

To describe the procedure for detection of antibody in the serum samples.

5. APPARATUS/TEST KIT/REAGENTS

Item	Reagents	Quantity
1	1 bottle of *Leptospira* Antigen:HRPO Conjugate (Preserved with gentamicin and Kathon)	3.8 mL
2	SNAP Device	5 or 10
Reagents contained in each device:		
Wash Solution (preserved with Kathon)		0.4 mL
Substrate Solution		0.6 mL
Other Components: transfer pipettes, sample tubes and reagent rack		

6. PROCEDURE

Sample Information

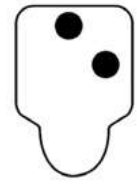
6.1 Samples must be at room temperature 18–25°C before beginning the test procedure.
6.2 Serum, either fresh or stored at 2–8°C for up to one week, can be used.
6.3 For longer storage, serum can be frozen (-20°C or colder) and then recentrifuged before use.
6.4 Hemolyzed or lipemic samples will not affect test results.
6.5 Allow all components to equilibrate at room temperature 18–25°C for 30 minutes before use. **Do not heat.**
6.6 Using the pipette provided, dispense **3 drops of sample** into a new sample tube.
6.7 Holding the bottle vertical, add **4 drops of conjugate** to the sample tube.
6.8 Cap the sample tube and mix it thoroughly by **inverting it 3–5 times.**
6.9 Place the device on a horizontal surface. Add the entire contents of the sample tube to the sample well, being careful not to splash the contents outside of the sample well. The sample will flow across the result window, reaching the activation circle in 30–60 seconds. Some sample may remain in the sample well.
6.10 When color FIRST appears in the activation circle, push the activator firmly until it is flush with the device body.
6.11 Read test result at 10 minutes

Note: Positive control may develop sooner, but results are not complete until 10 minutes.

7. RESULT INTERPRETATION

Positive Result

Any color development in the sample spots indicates the presence of anti-*Leptospira* antibody in the sample.
Note: A positive result indicates presence of antibodies to any of the following serovars: *Grippotyphosa, Canicola, Pomona, and Icterohaemorrhagiae*. The sample spot cannot differentiate between the four serovars.



Negative Result
Only positive control spot develops color.

Invalid Results

Background — If the sample is allowed to flow past the activation circle, background color may result. Some background color is normal. However, if colored background obscures test result, repeat the test.

No Color Development — If the positive control does not develop color, repeat the test.

8. WASTE DISPOSAL

Treat the samples as infectious agent and dispose properly after autoclaving or proper disinfection.

9. RISK ASSESSMENT

Leptospira can infect humans hence, utmost care should be taken while handling the specimens.

10. TROUBLESHOOTING

NA

11. REFERENCES

Curtis KM, Foster PC, Smith PS, et al. Performance of a recombinant LipL32-based rapid in-clinic ELISA (SNAP Lepto) for the detection of antibodies against *Leptospira* in dogs. Intern J Appl Res Vet Med. 2015;13 (3):182 –189.

SNAP test Lepto- Canine Leptospira Antibody test kit insert

TEST CATEGORIZATION FOR RAPID TESTS

Sl. No.	Procedure / SOP	DVL	SVL/TVH	RLDC/ NVH	NCAH
	Rapid antigen detection tests - Rapid Ag detection for Influenza Type A - Rapid Ag detection for H5 - Rapid Ag detection for rabies - Rapid Ag detection for IBD - Rapid Ag detection for NCD - Rapid Ag detection for PPR - Rapid Ag detection for CD - Rapid Ag detection for CPV Rapid antibody detection tests - Rapid Ab detection for FMD - Rapid Ab detection for EI - Rapid Ab detection for Bovine TB - Rapid Ab detection for Brucella	X	X	X	X