## TITLE: Alere Legionella

PRINCIPLE:

The Alere™ BinaxNOW® *Legionella* Urinary Antigen Card is an *in vitro* rapid immuno­chromatographic assay for the qualitative detection of *Legionella pneumophila* serogroup 1 antigen (*L. pneumophila* serogroup 1 antigen) in urine specimens from patients with symptoms of pneumonia. It is intended to aid in the pre­sumptive diagnosis of *Legionella* infection (Legionnaires’ Disease) caused by *L. pneumophila* serogroup 1 in conjunction with culture and other methods.

Rabbit anti-*Legionella pneumophila* serogroup 1 antibody, the patient line, is adsorbed onto nitrocellulose membrane. Control line antibody is adsorbed onto the same membrane as a second stripe. Both rabbit anti-*Legionella pneumophila* serogroup 1 antibodies and anti-species antibodies are conjugated to visualizing particles that are dried onto an inert fibrous support. The resulting conjugate pad and the striped membrane are combined to construct the test strip. This test strip and a well to hold the swab specimen are mounted on opposite sides of a hinged, book-shaped test device.

*L.* *pneumophila* serogroup 1 urinary antigen captured by immobilized anti-*L. pneumophila* serogroup 1 antibody reacts to bind conjugated antibody. Immobilized control antibody captures anti-species conjugate, forming the control line. A positive test result is read visually in 15 minutes. A negative Alere™ BinaxNOW® *Legionella* result, read in 15 minutes, indicates that *L. pneumophila* serogroup 1 antigen was not detected in the urine sample.

### PERSONNEL:

Medical Technologist

## REAGENTS AND EQUIPMENT:

1. **Reagents and Materials Provided**

|  |  |  |
| --- | --- | --- |
| **Component** | **Content** | **Quantity** |
| Test Device | Membrane coated with rabbit antibody specific for *Legionella pneumophila* serogroup 1 antigen and with control antibody is combined with rabbit anti-*Legionella pneumophila* serogroup 1 antigen and anti-species conjugates in a hinged test device. | 12/22 |
| Reagent A | Citrate/Phosphate with Tween® 20 and Azide | 1 |
| Swabs | Designed for use in the Alere™ BinaxNOW® *Legionella* Urinary Antigen Card. **Do Not Use Other Swabs**. | 12/22 |
| Positive Control Swab | Heat inactivated *L. pneumophila* dried onto swab. | 1 |
| Negative Control Swab | *L. pneumophila* negative swab. | 1 |

1. **Reagents and Materials not Provided**

Clock, timer or stopwatch

Standard urine collection containers

1. **Storage and Stability**

Store Test Kit at 2-30°C. The Alere™ BinaxNOW® *Legionella* kit and reagents are stable until the expiration dates marked on their outer packaging and containers

### SPECIMEN:

|  |  |
| --- | --- |
| A. Specimen: | Acceptable: Urine.  Unacceptable: Specimens collected from other sources. |
| B: Urine Container: | Acceptable: Standard Urine Collection Container. |
| C. Urine Preservative: | Acceptable: Boric Acid may be used as a preservative.  Unacceptable: Use of other preservatives. |
| D. Specimen Transport: | Transport in a leak proof container. |
| E. Specimen Storage: | Samples may be stored at room temperature if assayed within 24 hours. Alternatively, specimens may be stored at 2-8°C for up to 14 days or at -10° C to -20° C for longer periods before testing. |
| F. Handling Precautions: | Patient samples, controls, and test devices should be handled as though they could transmit disease. Observe established precautions against microbial hazards. |

## QUALITY CONTROL:

* 1. **Internal Procedural Controls**

The Alere™ BinaxNOW® *Legionella* has built-in procedural controls. For daily quality control, Alere suggests that you record these controls for each test performed.

* Positive Procedural Control – The pink-to-purple line at the Control position can be considered an internal positive procedural control. If capillary flow has occurred, this line will always appear.
* Negative Procedural Control – The clearing of the background color in the result window provides a negative background control. The background color in the window should be light pink to white with 15 minutes and should not interfere with the reading of the test result.
  1. **External Positive and Negative Controls**

Good laboratory practice suggests the use of positive and negative controls to ensure that:

• Test reagents are working; and

• The test is correctly performed.

Alere™ kits contain Positive and Negative Control Swabs. These swabs will monitor the entire assay. Test these swabs with each new ship­ment received and once every thirty days thereafter.

If the correct control results are not obtained, do not report patient results. Contact Technical Service at 1-877-866-9340 during normal business hours (EST).

## STEPWISE PROCEDURE:

**Patient Samples (and urine controls) Procedure:**

**Note: Use two (2) drops of Reagent A when testing liquid samples.**

Do not remove device from pouch until test sample has reached room temperature.

1. Allow reagents and devices to equilibrate to room temperature (15-30°C) before testing. Bring patient urine and or liquid urine controls to room temperature (59°-86°F, 15°-30°C). Remove device from its pouch **just before use** and lay flat.
2. Dip an Alere™ swab into the urine sample to be tested, completely covering the swab head. If the swab drips, touch swab to side of urine container to remove excess liquid.
3. There are two holes on the inner right panel of the device. Insert swab into the **BOTTOM** hole (swab well). Firmly push upwards so that the swab tip is fully visible in the **TOP** hole. **DO NOT REMOVE SWAB.**

4. Hold Reagent A vial vertically, ½ to 1 inch above the device. Slowly add two **(2) free** falling drops of **Reagent A** to the **BOTTOM** hole.

5. Immediately peel off adhesive liner from the right edge of the test device. Close and securely seal the device. Read result in window 15 minutes after closing the device.

Results read beyond 15 minutes may be inaccurate. However, some positive patients may produce a visible sample line in less than 15 minutes.

**Control Swab Test Procedure:**

**Note: Use six (6) drops of Reagent A for Control Swabs**

Remove device from the pouch just before use. Lay device flat and run test as follows:

1. There are two holes on the inner right panel of the device. Insert swab into the **BOTTOM** hole. Firmly push upwards so that the swab tip is fully visible in the **TOP** hole. **DO NOT REMOVE SWAB**.
2. Hold Reagent A vial vertically, ½ to 1 inch above the device. Slowly add **six (6)** free falling drops of **Reagent A** to the **BOTTOM** hole.
3. Immediately peel off adhesive liner from the right edge of the test device. Close and securely seal the device. Read result in window 15 minutes after closing the device. Results read beyond 15 minutes may be inaccurate. However, the positive control swab sample line may be visible in less than 15 minutes.

### REPORTING RESULTS:

The test is interpreted by the presence or absence of visually detectable pink-to-purple colored lines. A positive result will include the detection of both a patient and a control line, while a negative assay will produce only the control line. Failure of the control line to appear, whether the patient line is present or not, indicates an invalid assay.

**Negative Result**: A single pink-to-purple colored Control Line in the top half of the window indicates a presumptive negative result. This Control Line means that the detection part of the test was done correctly, but no *L. pneumophila* serogroup 1 antigen was detected.

**Positive Result:** Two pink-to-purple colored lines are visible in the window. This means that antigen was detected. Specimens with low levels of antigen may give a faint Sample Line. Any visible line is positive.

**Invalid Result:** If no lines are seen, or if just the Sample Line is seen, the assay is invalid. Invalid tests should be repeated. If the problem persists, contact Technical Service by phone at 877-866-9340.

### NOTES:

**Control swabs require six (6) drops of Reagent A. Patient samples require two (2) drops of Reagent A.**

1. Invalid Results, indicated by no control line, can occur when an insufficient volume of Reagent A is added to the test device. To insure delivery of an adequate volume, hold vial vertically, ½ - 1 inch above the swab well, and add drops slowly.
2. For *in vitro* diagnostic use.
3. If the kit is stored in a refrigerator, allow all kit components to equili­brate to room temperature (15-30°C) before use.
4. Leave test device sealed in its foil pouch until just before use. Do not use if pouch is damaged or open. Do not touch the reaction area of the test device.
5. Do not use kit past its expiration date.
6. Do not mix components from different kit lots.
7. Swabs in the kit have been approved for use with the Alere™ test. **Do not use other swabs.**
8. Solutions used to make the control swabs are inactivated using standard methods. However, precautions against microbial hazards should be observed.

**LIMITATIONS:**

* Sample types other than urine (e.g., plasma, serum, or other body fluids) that may contain *Legionella* antigen have not been validated with the Alere™ BinaxNOW® *Legionella*. The test cannot be used on environmental samples (i.e., potable water).
* The test will not detect infections caused by other *L. pneumophila* serogroups and by other *Legionella* species. A negative result does not exclude infection with *L. pneumophila* serogroup 1. Culture is recommended for suspected pneumonia to detect causative agents other than *L. pneumophila* serogroup 1 and to recover *L. pneumophila* serogroup 1 when antigen is not detected in urine.
* The diagnosis of Legionnaires’ disease cannot be based on clinical or radiological evidence alone. There is no single satisfactory laboratory test for Legionnaires’ disease. Therefore, to make an accurate diagnosis, culture results, serology, and antigen detection methods should be used in conjunction with clinical findings.
* Excretion of *Legionella* antigen in urine may vary depending on the individual patient. Antigen excretion may begin as early as 3 days after onset of symptoms and persist for up to 1 year afterwards. A positive Alere™ BinaxNOW® *Legionella* result can occur due to current or past infection and therefore is not definitive for infection without other supporting evidence.
* Performance of the Alere™ BinaxNOW® *Legionella* on diuretic urine has not been evaluated.
* The Alere™ BinaxNOW® *Legionella* has been evaluated on hospitalized patients only. An outpatient population has not been tested.

REFERENCES:

Alere BinaxNOW® *Legionella* Urinary Antigen Card CLSI + More Packet