## 1. PURPOSE

The Nitrazine (Phenaphthazine) paper test is a point of care test that is used to assess leakage of amniotic fluid due to premature rupture of the membranes or to aid in the diagnosis of bacterial vaginosis by measuring vaginal pH.

Nitrazine paper can be used to screen for the possible presence or leakage of amniotic fluid in the birth canal. If the Nitrazine test is positive, the provider follows up with a Fern Test, which is a Provider Performed Microscopy test.

pHizatest paper is a nitrazine indicator paper intended for in-vitro quantitative determination of pH in the 4.5 – 7.5 range.

## 2. PRINCIPLE

Nitrazine paper is impregnated with an indicator dye, Phenaphthazine. The color changes as pH changes, giving a range of colors from yellow through blue.

The Nitrazine pH test is a screen for presence of amniotic fluid.

- Negative test: paper remains yellow to olive green in color (pH 4.5 – 6.0)
- Positive test: paper turns blue green to deep blue in color (pH 6.5 – 7.5), suggesting the presence of amniotic fluid.

## 3. SCOPE

Qualified physicians, mid-level practitioners, and RNs may perform this test after appropriate training, competencies and after demonstrating that the colors utilized in the test can be discerned.

## 4. SPECIMEN REQUIREMENTS

Fresh vaginal pool specimen.
Specimen collection and labeling requirement: 2 unique patient identifiers

## 5. MATERIALS

Follow standard precautions and dispose all waste appropriately.

- pH Indicator paper and pH paper container.
  - pH paper should be stored at room temperature, out of direct sunlight.
  - Unopened pHizatest should be used by the printed expiration date.
  - Once opened, shelf life is 6 months.
- Disposable pipettes/tubes
- pH Nitrazine QC/Patient test Log sheet
- Sterile swab
- Gloves
- Fresh vaginal pool specimen collected according to clinical protocols.
  - The specimen is estimated to be stable for 2-5 minutes at room temperature.
- pH control, NIST certified buffer solution, 2 levels between 5.0 and 8.0.
  - Store buffers at room temperature.
  - Buffers are stable until the expiration date printed on the bottles.

| Item | Manufacturer | Vendor | How Packaged | One link number |
| --- | --- | --- | --- | --- |
| Buffer Solution, pH 5.00 (Certified) | Fisher Chemical SB102-1 | Fisher Scientific | bottle | 10353676 |
| Buffer Solution, pH 7.00 (Certified) | Fisher Chemical SB107-500 | Fisher Scientific | bottle | 10229740 |
| pHizatest Nitrazine Paper | Micro Essential Lab Cat# 934 | Fisher Scientific | Case of 10 paper rolls | 10352249 |

## 6. QUALITY CONTROL

Each lot of pHizatest has been verified for accuracy using NIST traceable standards. Dispose of expired rolls of nitrazine paper.

Once every week of patient testing, test two pH control solutions (pH 5.0 and pH 7.0) and record results on the pH QC log sheet.

1. Locate pH controls (pH 5.0 and 7.0).

2. Locate pH nitrazine paper. Tear off a strip of pH paper from the roll.

3. Dispense a drop of the pH 5.0 buffer solution onto the strip of paper (over sink or gauze pad observing standard precautions).

4. Immediately match the color of the pH paper with the closest color on the color chart supplied with the pH paper dispenser. Color comparison is recommended under a combination of florescent light and daylight.

5. Record pH reading on the QC Log sheet. The pH must be within 0.5 of the designated pH to be acceptable.

6. Repeat steps 1-3 with the pH 7.0 buffer solution.

7. If controls are out of range, do not test any patients until corrective action has been taken.

## 7. TROUBLESHOOTING

If QC testing is outside of the expected ranges use the following as a guideline to troubleshoot:

1. Repeat the procedure.
2. Check the buffer solution to make sure that it is not expired or contaminated and repeat using a new aliquot of pH buffer.
3. Repeat using a different Nitrazine test roll.
4. If controls are still out of range, or for any testing issue, call the Laboratory Point of Care Coordinator for assistance.

## 8. PROCEDURE

1. Verify patient's identity using two unique identifiers (name and MRN#).
2. Collect a fresh sample of vaginal pool fluid in a clean receptacle.
    - The specimen is estimated to be stable for 2-5 minutes at room temperature.
3. Apply aliquot of fluid to the nitrazine pH strip of paper.
4. Immediately match the color of the pH paper with the closest color on the color chart supplied with the pH paper dispenser. The color will fade after several minutes. Color comparison is recommended under a combination of florescent light and daylight.

5. Record results in the patient's chart.
    - Negative test: paper remains yellow to olive green in color (pH 4.5 – 6.0)
    - Positive test: paper turns blue green to deep blue in color (pH 6.5 – 7.5) suggesting the presence of amniotic fluid.

Note: Only a provider with current competency certification performs Fern Testing if the Nitrazine screen is positive.

## 9. RESULTS

Any resultant pH reading should be considered along with other laboratory and clinical findings to derive a final diagnosis.

If the nitrazine test is negative but the ferning is positive, there is probably a rupture of membranes.
If nitrazine test is positive and the ferning test is negative, a second specimen should be obtained.

- Record results in the Patient's Medical Record.
- Report results for Nitrazine test for vaginal pH as a numeric value.
- Report results for Nitrazine test for detection of amniotic fluid as:
    - pH 6.5 – 7.5 = positive
    - pH 6.0 and below = negative

## 10. LIMITATIONS

- Nitrazine screening test is highly sensitive but not very specific.
- Protect pH strips against exposure to acid or alkaline fumes.
- Color comparison recommended under a combination of fluorescent light and daylight. Base stock color of paper may vary from lot to lot, this will not affect the accuracy of the pH reading.
- The specimen is estimated to be stable for 2-5 minutes at room temperature after collection.
- Contamination with blood will interfere with reading. Bloody specimen should be read with caution, as it is difficult to interpret the color reaction.

- The Nitrazine paper measures pH generally within 1 pH unit between the range of 4.5 to 7.5 visually.
- False positive results may occur from specimen contamination due to heavy vaginal discharge, blood, cervical mucus, semen, alkaline urine, and soap. Note that cervical mucus, blood, urine, and glove powder have an alkaline ph.
- False negative results can result from prolonged rupture of membranes (longer than 24 hours) or when only a small quantity has leaked.
- Ferning test is less sensitive but more specific than the nitrazine test.

## 11. REFERENCES

1. Helper, Opal. 1966. *Manual of Clinical Laboratory Methods*, 4th Edition; Charles Thomas, Springfield, Illinois; pg 5.
2. Fisher Health Care 2002-2003. Fisher Scientific Company Santa Clara, CA; pg 780-781
3. Addison, Lois Anne. Obstetrical Ferning Test. *Laboratory Medicine*, July 1999, Volume 30, Number 7, p451.
4. Peter P. Toth, MD, PhD and A. Jothivijayarani, MD. Obstetrics: Premature Rupture of Membranes (PROM) *University of Iowa Family Practice Handbook*, 3rd Edition, Chapter 8
5. pHizatest Nitrazine paper product insert
6. McPherson & Pincus: Henry's Clinical Diagnosis and Management by Laboratory Methods, 23rd ed.
7. Policy adapted from Kaiser Permanente Medical Center, Oakland: Nitrazine Test, (E. Terry)
8. Policy adapted from Kaiser Permanente Medical Center, San Francisco: Nitrazine Screening Test. (A. Peretti) 5/2005.