510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k063295

B. Purpose for Submission:
New device

C. Measurand:
Urinary Nitrite and Leukocytes

D. Type of Test:
Qualitative

E. Applicant:
ACON Laboratories, Inc.

F. Proprietary and Established Names:
ACON UTI Urinary Tract Infection Test Strips

G. Regulatory Information:

1. Regulation section:
   21 CFR §862.1510, Nitrite (nonquantitative) test system
   21 CFR §864.7675, Leukocyte peroxidase test

2. Classification:
   Class I (meets the limitations of exemptions in 21 CFR 862.9 (c)(9)

3. Product code:
   LJX
   JMT

4. Panel:
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   See Indications for use below.

2. Indication(s) for use:
   The ACON UTI Urinary Tract Infection Test Strips are for the qualitative detection of Nitrite and Leukocytes in urine as an aid in the screening of urinary tract infection (UTI). It is intended for over-the-counter home use only.

3. Special conditions for use statement(s):
   For over-the-counter use. In the limitations section of the labeling, there are statements such as, "There is the possibility that this test may produce false results", "You may get a negative result if you have a UTI caused by bacteria that does not change nitrate to nitrite, when urine has not been held in the bladder for more than 4 hours, when taking antibiotics, or when your diet does not include nitrates" and "Consult your physician before making any medical decisions."

4. Special instrument requirements:
   None required; this is a self-contained visually read device.

I. Device Description:

ACON UTI Urinary Tract Infection Test Strips are plastic strips with a nitrite and a leukocyte reagent pad attached. They are packaged in a plastic bottle that contains desiccant and a separately packaged color chart. The reagent pads react with urine and change color. Test results are obtained by comparing the test strip to the color blocks printed on the color chart. The presence of nitrites in urine will turn the nitrite test pad pink to red. The presence of leukocytes will turn the leukocyte pad a shade between beige-pink and dark purple.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Bayer Multistix 10 SG Reagent Strips for Urinalysis

2. Predicate 510(k) number(s):
   k905396

3. Comparison with predicate:
   The predicate and the proposed device have the following similarities: assay format, test methodology, and assay matrix. The assays differ in their intended use setting (professional use in point-of care setting versus over-the-counter) and their intended use (the predicate device also detects additional analytes that may provide information regarding the status of carbohydrate metabolism, kidney and liver function, acid-base balance in addition to information about urinary tract infections).

K. Standard/Guidance Document Referenced (if applicable):

None referenced.

L. Test Principle:

Nitrite: Gram negative bacteria in urine convert nitrate to nitrite. In an acidic medium, nitrite reacts with p-arsanilic acid to form a diazonium compound. The diazonium compound in turn couples with 1-N-(1-naphthyl)-ethylenediamine to produce a pink color. Nitrite is not detectable in normal urine therefore a consistent pink to red color evidences nitrite in urine.

Leukocytes: This test reveals the presence of granulocyte esterases in urine. If esterases are present, they cleave a derivatized pyrazole amino acid ester to liberate derivatized hydroxy pyrazole. This pyrazole reacts with a diazonium salt to produce a beige-pink to purple color. Normal urine is usually negative for leukocyte esterases. Therefore, a color change to +, ++, or +++ may have clinical significance; a color change to 'trace' (±) is considered clinically negative for leukocytes but may have clinical significance if the user gets repeated trace results.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*
   Reproducibility of ACON UTI Test Strips was tested by professional users using a negative control solution and a moderately positive control solution (positive for nitrites, 2+ for leukocytes). Three lots of strips were used. Each control was tested in duplicate by five operators per day for ten days (total strips tested = 600). Results were read by comparing color reaction to the color blocks on the canister label according to the package insert instructions. Test results agreed with the expected results each day for each lot tested at both levels tested.

   The ability of lay users to read the test results is demonstrated in Section 3.c. "Other clinical supportive data" below.

   b. *Linearity/assay reportable range:*
   Not applicable. These tests are intended for qualitative use.

   c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
   No traceability was provided.

   Stability testing protocol and acceptance criteria were provided. The sponsor's accelerated stability testing suggests that the strips will have a 24 month shelf life when stored unopened at room temperature. Real time stability testing is currently underway.

   d. *Detection limit:*
   See Assay Cutoff section below.

   e. *Analytical specificity:*
   Potentially interfering endogenous and exogenous substances at the concentrations in the table below were added to normal urine, urine spiked with leukocyte positive urine (125 Leu/uL), and nitrite positive urine (1.0 mg/dL). All tests with normal (negative) urine samples returned negative results except that sodium nitrite produced a positive result for the nitrite test. All tests with positive urine samples returned positive results except albumin, ascorbic acid, glucose, sodium bicarbonate and riboflavin. These compounds were tested further as described below.

ACON UTI Test Strips: Compounds Tested for Potential Interference

Potential Interferent	Tested Concentration (mg/dL)		Potential Interferent	Tested Concentration (mg/dL)
Albumin	1000		Lactose	1
Ammonium Chloride	200		Lithium Acetoacetate	250
Ascorbic Acid	200		Oxalic Acid	70
Bilirubin	4		Phenolphthalein	1200
Calcium Chloride	80		Potassium Chloride	1200
Citric Acid	65		Riboflavin	10
Creatinine	600		Sodium Chloride	1800
Fructose	1.2		Sodium Nitrate	0.3
Galactose	0.5		Sodium Nitrite	0.1
Glucose	4100		Sodium Phosphate	500
Glycine	450		Theophylline	100
Hemoglobin	5		Urea	4000

A series of experiments were performed to determine if common urine components, including the compounds that caused positive test results in the study above, would affect the performance of the ACON UTI test strips. The components tested with negative urine, samples spiked with nitrite at 0.05, 0.1, and 1.0 mg/dL, and samples spiked with leukocytes at 15, 70, 125, and 500 Leu/uL. The concentrations of the interferents that caused positive test results above are generally much higher than would be found in urine of typical users. The labeling includes cautions that ascorbic acid and riboflavin may interfere with the test. The results of these studies are summarized in the following table:

Interferents: ACON UTI Test Strips

Interferent	**Effect on Nitrite Test**		**Interferent**	**Effect on Leukocyte Test**
Specific Gravity ≥ 1.030	2/5 positive at 0.05 mg/dL		Specific Gravity ≥ 1.025	**negative results** at 15 cells/uL
Ascorbic Acid ≥ 30 mg/dL	0/5 positive at 0.05 mg/dL		Ascorbic Acid	no effect at tested concentrations

Interferent	Effect on Nitrite Test		Interferent	Effect on Leukocyte Test
pH	no effect at tested concentrations		pH ≥ 8.0	1 of 5 tests read positive at 15 cells/uL
Riboflavin ≥ 10 mg/dL	0/5 positive at 0.05 mg/dL		Riboflavin ≥ 10 mg/dL	1/5 negative at 15 cells/uL
Sodium Bicarbonate ≥ 1000 mg/dL	0/5 positive at 0.05 mg/dL		Sodium Bicarbonate	no effect at tested concentrations
Glucose	no effect at tested concentrations		Glucose ≥ 2000 mg/dL	lower than expected readings at all tested concentrations
Albumin	no effect at tested concentrations		Albumin ≥ 1000 mg/dL	0/5 positive at 0.05 mg/dL

The effect of the temperature of the test solution, the length of time the strip was in contact with the urine sample ('dip time') and the test read time after dipping were also evaluated. Calibrator solutions (negative, low positive and strongly positive) were used in the tests. Storage of the calibrator solutions at temperatures ranging from 4°C to 45°C for 30 minutes before using them to test the strips did not affect the strip's detection of negative or positive samples. Dip times of the recommended 2 seconds up to 10 seconds did not alter the expected result of the test at any read time tested (15 seconds to 30 minutes). Dip times of less than two seconds only affected the test result when the leukocyte test was read at 15 or 30 seconds (recommended read time is two minutes). Read times greater than five minutes caused the negative nitrite and leukocyte tests to read 'positive' and the positive leukocytes to read one color block higher than expected. The labeling instructs users to read the nitrite test at 1 minute and the leukocyte test at 2 minutes but not to read either test beyond 3 min.

f. Assay cut-off:

The sensitivity of each test was assessed by testing a range of urine samples spiked to known concentrations. The sponsor defined the minimum sensitivity level as the lowest level at which over 67% of the test results show a color change equivalent to the first color block on the color chart, i.e a uniform pink for the nitrite test and the beige-pink of the trace block for the leukocyte test. Three lots of strips were tested. For three consecutive days each sample was tested 21 times by three operators (7 strips per operator per day). A total of 189 strips were tested at each concentration (3 operators x 3 days x 7 strips x 3 lots). Results were read by comparing color reactions on the strip to the color chart according to the package instructions. Results are shown in the tables below:

Nitrite Sensitivity: ACON UTI test strips

Nitrite Conc. (mg/dL)	Negative (yellow)	Positive (pink)	% Positive
0.025	189	0	0
0.05	3	186	98.4 %
0.06	0	189	100 %
0.08	0	189	100 %
0.1	0	189	100 %

Leukocyte Sensitivity: ACON UTI test strips

Leukocyte Conc. (Cells/uL)	0 Cells/uL	Trace * (15Cells/uL)	+ (70Cells/uL)	+ + (125Cells/uL)	% Agreement
3.75	189	0	0	0	0
7.5	86	103	0	0	54
9	8	181	0	0	96
12	0	189	0	0	100
15	0	189	0	0	100

* "Trace" in the ACON UTI strip is claimed as equivalent to 9 – 15 cells/uL; "trace" in the Bayer predicate is claimed as equivalent to 5- 15 cells/uL. In clinical interpretation of both tests a reading of "Trace" is negative for leukocytes. The labeling instructs users to read the test as positive when the leukocyte result is ≥ + .

The sponsor claims a minimum analytical detection limit of 0.05 mg/dL for the nitrite test and 9 – 15 cells/uL for the leukocyte test.

2. Comparison studies:
   a. *Method comparison with predicate device:*
      See clinical studies section below.

   b. *Matrix comparison:*
      Not applicable; this device is for use with urine samples only.

3. Clinical studies:
   a. *Clinical Sensitivity:*
      Not applicable

   b. *Clinical specificity:*
      Not applicable

   c. Other clinical supportive data (when a. and b. are not applicable):
      One hundred symptomatic* inexperienced lay users tested their own freshly collected urine specimens with the ACON UTI Urinary Tract Infection Test Strips. Their results were compared to results obtained by laboratory

personnel using the ACON UTI Urinary Tract Infection Test Strips and the predicate tests. Results are shown below:

Leukocyte Results: ACON/Lay User vs. ACON/Professional

	Professional				
Lay User	Neg	Trace (±)	+	++	+++
Neg	70				
Trace		4			
+		2	8	2	
++				12	
+++					2
% Exact Agreement	**96%** (96/100)				
% Agreement (±One Color Block)	**100%** (100/100)				

Nitrite Results: ACON/Lay User vs. ACON/Professional

	Professional	
Lay User	Nitrite (-)	Nitrite (+)
Nitrite (-)	96	0
Nitrite (+)	0	4
% Exact Agreement	**100%** (100/100)	
% Agreement (± One Color Block)	**100%** (100/100)	

Leukocyte Results: ACON/Professional vs. Bayer/Professional

	Bayer				
ACON	Neg	Trace (±)	+	++	+++
Neg	69	1			
Trace		6			
+			0	8	
++			1	10	3
+++				1	1
% Exact Agreement	**86%** (86/100)				
% Agreement (±One Color Block)	**100%** (100/100)				

Nitrite Results: ACON/ Professional vs. Bayer/Professional

	Bayer	
ACON	Nitrite (-)	Nitrite (+)
Nitrite (-)	96	0
Nitrite (+)	0	4
% Exact Agreement	**100%** (100/100)	

***** UTI symptoms included at least one of the following: burning or pain during urination, frequent urination, fever, back or groin pain, or cloudy, dark or bloody urine, or bad smelling urine. There were no follow up culture results.

Because there were so few nitrite positive samples in the clinical study, the sponsor recruited 40 lay-users to participate in a readability study with spiked urine specimens at different concentrations of leukocyte and nitrite:

Spiked Sample #	Spiked Concentrations	
	Leukocyte	Nitrite
1	Negative	Negative
2	Trace	Negative
3	+	1st positive color block
4	+++	2nd positive color block

Each participant tested a panel of 4 coded and blinded spiked urine samples with the ACON test using the package insert. The same panel of spiked urine samples was also tested at the same time by a professional with the ACON test and the predicate. The test results are summarized in the following tables.

ACON Test: Lay User vs. Expected	Spiked Sample #1		Spiked Sample #2		Spiked Sample #3		Spiked Sample #4	
	Leu (-)	Nit (-)	Leu (trace)	Nit (-)	Leu (+)	Nit (+)	Leu (3+)	Nit (+)
% Agreement (Exactly Match)	100%	100%	83% (33 "trace", 4 "+" & 3 "-")	100%	85%* (34 "+" & 6 "++")	100%	98%* (39 "+++" & 1 "++")	100%
% Agreement (± One Color Block)	100%	100%	100%	100%	100%	100%	100%	100%

* These disagreements of the readings do not result in a change of the interpretation of the test result.

ACON Test: Prof. vs. Expected	Spiked Sample #1		Spiked Sample #2		Spiked Sample #3		Spiked Sample #4	
	Leu (-)	Nit (-)	Leu (trace)	Nit (-)	Leu (+)	Nit (+)	Leu (3+)	Nit (+)
% Agreement (Exactly Match)	100%	100%	100%	100%	100%	100%	100%	100%

Predicate Test: Prof. vs. Expected	Spiked Sample #1		Spiked Sample #2		Spiked Sample #3		Spiked Sample #4	
	Leu (-)	Nit (-)	Leu (trace)	Nit (-)	Leu (+)	Nit (+)	Leu (3+)	Nit (+)
% Agreement (Exactly Match)	100%	100%	100%	100%	100%	100%	100%	100%

ACON Test: Lay User vs. Prof.	Spiked Sample #1		Spiked Sample #2		Spiked Sample #3		Spiked Sample #4	
	Leu (-)	Nit (-)	Leu (trace)	Nit (-)	Leu (+)	Nit (+)	Leu (3+)	Nit (+)
% Agreement (Exactly Match)	100%	100%	83% (33 "trace", 4 "+" & 3 "-")	100%	85%* (34 "+" & 6 "++")	100%	98%* (39 "+++" & 1 "++")	100%
% Agreement (± One Color Block)	100%	100%	100%	100%	100%	100%	100%	100%

* These disagreements of the readings do not result in a change of the interpretation of the test result.

ACON vs. Bayer by Professional	Spiked Sample #1		Spiked Sample #2		Spiked Sample #3		Spiked Sample #4	
	Leu (-)	Nit (-)	Leu (trace)	Nit (-)	Leu (+)	Nit (+)	Leu (3+)	Nit (+)
% Agreement (Exactly Match)	100%	100%	100%	100%	100%	100%	100%	100%

4. Clinical cut-off**:**
   Not applicable.

5. Expected values/Reference range:
   Normal urine is usually negative for leukocytes and nitrites.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10..

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.