URITOP®+

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URINE TEST STRIPS FOR THE QUALITATIVE AND SEMI-QUANTITATIVE DETECTION OF SEVERAL BIOCHEMICAL PARAMETERS IN URINE

For professional in vitro diagnostic use

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1 I INTENDED USE

The URITOP®+ reactive strips for urine analysis are rigid plastic strips on which several areas of reagents are fixed. The test is intended for the qualitative and semi-quantitative detection in human urine of several parameters: Urobilinogen, Glucose, Bilirubin, Ketones (Acetylacetic Acid), Specific Gravity, Blood, pH, Protein, Nitrite, Leukocytes, Ascorbic Acid, Microalbumine and Creatinine. The test is intended for professional use *in vitro* diagnostic use only.

Refer to the table below for the relevant parameter(s) according to the product references:

Product name	Reference	Parameters
URITOP®+ 5	1040007	Glucose, ketones, blood, protein, pH
URITOP®+ 7	1040008	Glucose, specific gravity, blood, leukocytes, protein, nitrites, pH
URITOP®+ 11	1040010	Glucose, bilirubine, ketones, specific gravity, blood, leukocytes, protein, urobilinogen, nitrites, pH, ascorbic acid
URITOP®+ 13	1040011	Glucose, bilirubine, ketones, specific gravity, blood, leukocytes, protein, urobilinogen, nitrites, pH, ascorbic acid, microalbumin, creatinine

2 I TEST PRINCIPLE

Urine undergoes many changes during the stages of disease or body dysfunction before blood composition is affected significantly. Urine analysis is a useful procedure indicator of good health or disease and is part of a medical database. Reactive strips can be used in a general health examination and allow the diagnosis and monitoring of systemic and metabolic diseases that affect kidney function, endocrinological diseases and diseases or disorders of the urinary tract infection.

Strips may be read visually by comparison of test paper attached to the plastic strip with the color chart blocks printed on the vial label. They can also be read instrumentally, using URITOP®300 or MINI URITOP® readers.

3 I MATERIALS

Materials provided

- 1 bottle of 100 strips with a color chart printed on the bottle.
- 1 instructions for use.

Materials required but not provided

- Timer.
- Container for sample collection.

4 I STORAGE AND STABILITY

- Keep the closed bottle at room temperature or refrigerated between 2-30°C (36-86°F). Do not store strips in the refrigerator or freeze.
- Store away from moisture and sunlight.
- When stored in the original, unopened bottle, the strips are stable until the expiration date indicated on the bottle.
- **Use the strips within 6 months of opening the bottle.** Beyond this time, there is a risk of discolouration or discolouration of the test areas due to exposure to moisture or light. Do not remove the desiccant
- Open the bottle just before performing the test. Close the vial tightly immediately after each use. Keep the vial tightly closed between uses.

5 I PRECAUTIONS

- For *in vitro* use by professionals only.
- Do not touch the test areas on the test strips.
- Discoloured or darkened test areas may indicate deterioration. If this is the case, or if results are uncertain or inconsistent with expectations, check the expiration date and use known negative and positive controls.
- Wear a gown, gloves and eye protection when performing the test. Avoid contact with skin and eyes.
- Do not eat, drink or smoke when handling specimens and testing.
- Specimens may be contaminated with infectious agents. Treat materials in direct contact with specimens as contaminated. When performing the test, take precautions when handling infectious materials.
- The test, once used, should be disposed of according to local procedures.

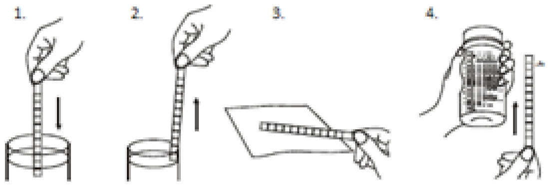
6 I SAMPLE COLLECTION AND STORAGE

- Collect the freshly emitted urine in a clean, dry container to allow total immersion of the strip. Do not add preservatives. If urine samples are not collected in clean containers, positive leukocyte results may be observed due to urine contamination.
- Nitrite, bilirubin, urobilinogen: The use of fresh morning urine is recommended for optimum results, as these parameters are unstable in light.
- It is preferable to test the sample within one hour after urination, having homogenised the sample well, but do not centrifuge it.
- If the test cannot be carried out within one hour (stored at room temperature), store the urine for a maximum of 4 hours at 2-8°C and allow it to return to room temperature before testing. Do not freeze.
- Urine stored at room temperature changes its pH due to bacterial growth, which interferes with the determination of proteins.
- Hygiene products containing chlorhexidine may affect the protein results if the sample is contaminated.

7 I TEST PROCEDURE

The described procedure must be followed strictly to obtain a reliable result. Do not compare the strips with the colour chart before the strips are immersed in the urine.

1. Immerse the strip for 2 seconds (maximum) in the urine, wetting all the test areas completely (see Figure 1 below).
2. Remove the strip by sliding the back of the strip against the walls of the container to remove excess urine (see Figure 2 below). Take care not to let the test areas of the strip come into contact with the walls of the container during this step.
3. Hold the strip in a horizontal position and contact the edge of the strip with absorbent paper (e.g. paper towel) to remove excess urine to avoid mixing chemicals from adjacent test areas (see Figure 3 below). Excessive urine on the strip can create chemical interactions between two adjacent test areas and lead to incorrect results.
4. Visual Reading: Results are read at 60 seconds (except leukocytes at 90-120 seconds): compare the colours of the test areas obtained against the colour chart attached to the vial under good lighting conditions (see Figure 4 below). When reading, keep the strip horizontal to prevent mixing of the different chemical reagents which may occur in case of excess urine.



Reading with the URITOP®300 Reader or the MINI URITOP®: Follow the instructions in the reader's manual carefully. The reader will automatically read each reactive zone at the given time

8 I RESULTS INTERPRETATION

The results are obtained by direct comparison of the colour blocks printed on the colour chart. The colour blocks represent nominal values; actual values will vary closely to the nominal values. In the event of unexpected or questionable results, the following steps are recommended: confirm that the strips were tested before the expiry date printed on the bottle label or airtight pouch, compare the results with the positive and negative controls, and retest with a new strip. If the problem persists, stop using the tests and contact BIOSYNEX.

9 I CHEMICAL PRINCIPLES, INGREDIENTS AND LIMITATIONS

Blood

Chemical principle: The test is based on the pseudo-peroxidase activity of the heme entity of haemoglobin or myoglobin. The chromogen is oxidised by a hydroperoxide in the presence of haem and causes a colour change from yellow to blue.

Reagents: Cumene Hydroperoxide 12 mg, o-Tolidine 35 mg.

Expected values: Normally no trace of haemoglobin is detectable in the urine (0.010 mg/dL; 3 erythrocytes/µL). The presence of haemoglobin in the urine indicates either kidney disease or a disorder of the urinary tract. Blood is often found in the urine of women during menstrual periods. The significance of the presence of traces of haemoglobin can vary according to the patient and the clinical situation. A clinical evaluation is necessary to assess each individual case. The test is highly sensitive for the detection of haemoglobin (however, it loses sensitivity when the red blood cells are intact). It is a complement to the microscopic examination.

Detection limit: 10 erythrocytes/µL. The test is more sensitive in the presence of free haemoglobin or myoglobin than in the presence of intact erythrocytes. Sensitivity is reduced in the presence of urine with a high specific gravity or containing ascorbic acid. The presence of green spots on the reactive zone indicates the presence of intact erythrocytes in the urine.

Limitation: A high specific gravity or high protein content can reduce the reactivity of the test. The presence of microbial peroxidase associated with a urinary tract infection can lead to a false positive result. Ascorbic acid concentrations greater than 30 mg/dL may cause false negative results when haemoglobin is present in trace amounts. It is important to resuspend red blood cells prior to testing. Do not use collection containers containing traces of oxidising substances (e.g. bleach). Consider the risk of interference linked to menstruation, leucorrhoea or catheterisation.

Bilirubin

Chemical principle: Azo-coupling reaction of bilirubin with a diazonium salt in an acidic medium to form an azo compound. Colouring varies from beige to light pink.

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Reagents: Sodium nitrite 0.733 mg, 2,4-dichlorobenzene diazonium 2.3 mg, Sulfosalicylic acid 25 mg.
Expected values: Normally bilirubin is not detectable in urine even with the most sensitive methods. The presence of traces of bilirubin is sufficient to trigger further investigations.
Detection limit: 1 mg/dL.
Limitation: Drug metabolites such as pyridium or selenium, which stain at low pH, may give false positive results. Indican (indoxyl sulfate) can produce a yellow-orange to red colouration which may interfere with the reading of a positive or negative bilirubin result. Ascorbic acid (concentration >30 mg/dL), may give false negative results. The bilirubin test should be performed on freshly emitted urine avoiding prolonged exposure to light.

Urobilinogen
Chemical principle: The reaction is based on the Ehrlich reaction. The colouring varies from light orange pink to dark pink.
Reagents: 4-Methoxybenzenediazonium 2.9 mg.
Expected values: The normal urinary concentration of urobilinogen is between 0.1 and 1.0 Ehrlich units/dL. If the result is above a concentration of 2.0 mg/dL, the patient and his urine will need to undergo further tests.
Detection limit: The test can detect urobilinogen concentrations as low as 0.1 mg/dL. However, the absence of urobilinogen in the urine cannot be asserted. In patients with elevated urobilinogen levels, the results are closely correlated with the Watson-Schwartz spectrophotometric method.
Limitation: The absence of urobilinogen in the urine cannot be asserted. The reactive zone may react with certain substances such as p-aminosalicylic acid. Drugs containing azogantrisine may produce a masking golden discolouration. The test does not detect porphobilinogen. The test for urobilinogen should be performed on freshly emitted urine avoiding prolonged exposure to light.

Ketones
Chemical principle: Legal's test-nitroprusside reaction. Acetylacetic acid in an alkaline medium reacts with a nitroferricyanide which produces a colour change from beige to purple.
Reagents: Sodium nitroprusside 23.0 mg.
Expected values: Ketones are not detectable with this reagent in normal urine.
Detection limit: 5 - 15 mg/dL. Urine with high specific gravity and low pH can react. A clinical evaluation is therefore necessary in the presence of traces of ketones obtained with this reagent.
Limitation: A positive result (trace or less) may occur with urine that is highly pigmented or contains significant acmounts of levadopa metabolites. High SG levels and low urine pH may cause false positive results. Phenosulfonphthalein may also cause false positive results. Detectable levels of ketones may occur in the urine during physiological stress such as fasting, pregnancy, exercise in ketoacidosis, starvation, or in the presence of disturbances in carbohydrate or lipid metabolism. Ketone bodies can appear in the urine in large quantities before their levels rise in the serum. Severe bacteriuria can negate the test.

Glucose
Chemical principle: Glucose oxidase catalyses the oxidation of glucose to form hydrogen peroxide. The hydrogen peroxide thus formed oxidizes a chromogen located on the reactive zone by the action of peroxidase.
Reagents: Glucose oxydase 430 U, Peroxydase 200 U, o-Tolidine 12 mg
Expected values: A low concentration of glucose (close to 30 mg/dL) is normally considered normal. Glucose is normally absent in the urine. However, it is eliminated in small quantities by the kidney. A concentration of 50 mg/dL or more can be considered abnormal, especially if repeated.
Detection limit: The detection limit of the test strip is 50 mg/dL glucose. The reading range is up to 1000 mg/dL. The test is highly specific for glucose. The test area does not react with lactose, galactose, fructose or reducing metabolites of salycilates and nalidixic acid.
Limitation: High specific gravity (>1,020) with high pH and ascorbic acid (concentration > 40mg/dL) can cause false negatives in urine with low glucose concentration. Do not use collection containers containing traces of oxidising substances (e.g. bleach).

Protein
Chemical principle: It is based on the protein error of pH indicator papers. When the pH is kept constant in a buffer system, the pH indicator releases the H+ ion in the presence of proteins, which causes the colour to change from yellow to blue-green.
Reagents: Tetrabromophenol blue 0.34 mg
Expected values: Urine usually contains low levels of protein (<20 mg/dL). Only the persistent and elevated presence of urinary protein indicates kidney disease or urinary tract infection. Persistent presence of trace or higher levels of proteinuria indicates significant proteinuria. In this case, further investigations should be carried out to assess the significance of the result.
Detection limit: The detection limit of the test is 15-30 mg/dL protein.
Limitation: False positive results may occur in strongly basic urine (pH 9). The interpretation of the results is also problematic in turbid urine. Do not use collection containers containing traces of oxidising substances (e.g. bleach). Avoid toilets cleaned with quaternary ammonium salts (risk of false positives).

Nitrite
Chemical principle: The test is based on a nitrite diazotization reaction with an aromatic amine to produce a diazonium salt. This is followed by a coupling reaction of this diazonium salt with an aromatic compound located in the reaction zone. The azo compound formed will produce a colour change from white to pink.
Reagents: P-arsanilic acid 4.5 mg
Expected values: Nitrites are usually absent in urine. Their presence indicates the presence of bacteria that may be responsible for infections of the kidneys, urethra, ureter or bladder.
Detection limit: The detection limit is 0.05 mg/dL. Comparison of the colour obtained against a white background can help in the detection of small amounts of nitrite that might otherwise go undetected. The test is specific for nitrite and does not react with other substances normally excreted in the urine.
Limitation: Ascorbic acid (>30 mg/dL) can cause a false negative result with low levels of nitrite in the urine (<0.03 mg/dL). A negative result does not mean that the patient is free of bacteria. Pink spots or pink staining at the corners should not be interpreted as a positive result. Any uniform pink staining indicates the presence of at least 10^5 bacteria/mL, but the intensity of the staining is not proportional to the bacteriuria. Negative results occur in urinary tract infections caused by an organism that does not contain nitrate reductase. The urine sample should not be tested more than 4 hours after collection, otherwise a reduction of nitrate to nitrite will occur. Urine stored over a long period of time can give false negative results and false positive results in case of bacterial contamination for example. Taking nitrate derivatives can lead to false positive results.

Leukocytes
Chemical principle: The reactive zone contains an indoxyl ester and a diazonium salt. The diazonium salt will form an aromatic derivative in the presence of leukocyte esterase, which by a copulation reaction will cause a coloured derivative to appear, which turns from beige to violet.
Reagents: Indole amino acid ester 1.3 mg.
Expected values: Leukocytes are normally not detectable in urine. The result must be interpreted according to the clinical context, especially in the presence of traces.
Detection limit: The test can detect the presence of trace amounts of leukocytes at 20~25 leukocytes/µL.
Limitation: The result may not always correlate with the microscopic leukocyte count. High concentration of glucose, high specific gravity, high presence of albumin, high concentration of formaldehyde, or the presence of blood may decrease the intensity of the result. High concentrations of oxalic acid or the presence of traces of oxidising or acidic agents can negatively affect the result.

pH
Chemical principle: Double indicator system. Methyl red and bromothymol blue are used to generate a colour change from orange to green and blue on a scale from 5.0 to 9.0.
Reagents: Methyl Red 0.05 mg, Bromothymol Blue 0.5 mg.
Expected values: Urine pH usually ranges from 5 to 9. The urinary pH is an important indicator of certain metabolic situations related to the functioning of the kidney and the gastrointestinal and respiratory systems.
Detection limit: The test measures pH values between 5-9 with an accuracy of one unit.
Limitation: Excessive amounts of urine on the strip due to improper handling can cause the acid reagent on the protein detection zone to move to the pH measurement zone and give an acidic pH result in the presence of neutral or alkaline urine. This phenomenon is called "run-over". The pH measurement should be carried out on freshly emitted urine because bacterial growth causes the urine to become alkaline.

Specific gravity
Chemical principle: Ionic elements in urine release protons that lower the pH value and cause a colour change in the presence of bromothymol blue, which changes from blue-green to yellow-green.
Reagents: Bromothymol blue 0.5 mg, Poly (methyl vinyl ether/maleic acid) anhydrous 140.5 mg.
Expected values: The density of adult urine ranges from 1.001 to 1.035. The first morning urine has a specific gravity between 1.015 and 1.025. The specific gravity of newborn urine ranges from 1.002 ~1.004. In the case of severe kidney damage, the specific gravity is set at 1.010, which corresponds to the density of the glomerular filtrate.
Detection limit: The test allows the determination of densities between 1.000, 1.005, 1.010, 1.015, 1.020, 1.025, 1.030. Alkaline buffered urine may give default results.
Limitation: Strongly alkaline urine may cause a decrease in the result, whereas strongly acidic urine causes a slight increase in the result. A high specific gravity can be obtained in the presence of moderate amounts of protein. The specific gravity also increases in the presence of glucose. A pH > 6.5 may result in a default value for the specific gravity.

Ascorbic acid
Chemical principle: The reactive zone contains the discolouration of the Tillmann reagent. The presence of ascorbic acid changes the colour from green to green-yellow.
Reagents: sodium salt 2.6-dichloro indophenol 0.8 mg.
Expected values: For an average daily intake of 30-80 mg, a secretion of 20-30 mg/day of ascorbic acid is observed.
Detection limit: Low concentrations of ascorbic acid (up to 50 mg/dL) in urine may cause interference with samples containing low concentrations of glucose, blood and bilirubin. Concentrations of 200 mg/dL or more can cause strong interference. If ascorbic acid is detected in the urine, reexamine after 24 hours without ingesting ascorbic acid.
Limitation: No interference is known.

Microalbumin
Microalbuminuria is an abnormal increase in the rate of urinary albumin excretion. It is often one of the first signs of kidney disease or damage that can lead to kidney

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failure. Patients with high blood pressure or diabetes are at greater risk of microalbuminuria and kidney disease. Microalbuminuria refers to the urinary excretion of very small amounts of albumin in the urine.
Chemical principle: This test is based on a reaction with sulfonephthalein. At a constant pH, albumin binds with the sulfonephthalein and develops a blue colour. The colour ranges from pale green to turquoise blue.
Reagents: Sulphonephthalein 0.1 mg, Citric acid 30 mg
Expected values: Urine usually contains albumin below 2 mg/dL. A result in the range of 3~30 mg/dL indicates microalbuminuria.
Detection limit: 3 mg/dL (albumin).
Limitation: Large amounts of hemoglobin (≥ 5 mg/dL), urine with blood, highly alkaline urine (pH > 8), or with a disinfectant including a quaternary ammonium compound, may cause false positive results.

Creatinine

Creatinine is a product of muscle metabolism and the excretion of creatinine in the urine is usually constant. The measurement of creatinine is used in the diagnosis and treatment of kidney disease, for monitoring kidney dialysis, and as a basis for measuring other urine analytes. Although the concentration (or dilution) of urine varies throughout the day, the urinary creatinine level is relatively stable, allowing its measurement to be used as a corrective factor in random urine samples.
Chemical principle: The test is based on a reaction of creatinine with a colour-metallised complex. Under alkaline conditions, creatinine reacts with the metal-coloured complex to form a brown-violet coloured complex.
Reagents: Picric Acid 0.3 mg, Borax 20 mg.
Expected values: Urine usually contains a creatinine level of 10~300 mg/dL. Alteration of the urine sample or severe kidney failure can cause very low creatinine results.
Limitation: Medications containing azo dyes, nitrofurantoin, riboflavin may affect the results and give dark, brown-colored urine.

Microalbumin to Creatinine ration

When albumin and creatinine are measured simultaneously from a single urine sample, the albumin-creatinine ratio (ACR) can be determined. The ACR is the most practical test recommended by the American Diabetes Association for screening for microalbuminuria.
Chemical principle: The following table is used to obtain the Microalbumin to Creatinine ratio.

		Creatinine mg/dL (mmol/L)				
		10 (0.9)	50 (4.4)	100 (8.8)	200 (17.7)	300 (26.5)
Microalbumin mg/dL (mg/L)	1 (10)	*			Normal	
	3 (30)					
	8 (80)	Highly abnormal		Abnormal		
	15 (150)					

* Dilute the sample to confirm the ratio result. Repeat the test with a new sample, preferably during the first urine in the morning.

Example :

Reading	Reported result	Creatinine result	Microalbumin to Creatinine Ration
Microalbumin = 15 mg/dL Protein = 30 mg/dL	30 mg/dL	100 mg/dL	Abnormal
Microalbumin = 8 mg/dL Protein = Negative	8 mg/dL	300 mg/dL	Normal

Microalbumin/Creatinine ratio interpretation:

	Normal	Abnormal	Highly Abnormal
Conc. (mg/g)	< 30	30-300	> 300
Conc.(mg/mmol)	< 3.4	3.4-33.9	> 33.9

Expected values: Microalbumin is normally present in the urine at concentrations below 30 mg albumin/g creatinine. A result in the range of 30~300 mg/g (Abnormal) indicates microalbumin and a result > 300 mg/g (Highly Abnormal) indicates clinical albuminuria.
Limitations: A low microalbumin level (10 mg/L) in highly diluted urine (creatinine result 10 mg/dL) may indicate a microalbumin concentration below the sensitivity limit. In this case, it is best to test a new sample, preferably in the first morning urine to avoid interference.

10 I QUALITY CONTROL

Controls are not supplied in this kit. The positive control supplied by BIOSYNEX under reference 6040001 has been designed to validate the URITOP®+ product range. Any other commercial control has not been validated and is therefore not recommended.
It is recommended, according to Good Laboratory Practice, to carry out a control on each new batch or each new delivery to confirm the procedure and check performance. Each laboratory must define its own quality control system.

11 I PERFORMANCE CHARACTERISTICS

Performance has been based on clinical studies that depend on several factors: variation in colour perception; the presence or absence of inhibiting factors found in urine and the laboratory conditions in which the product is used (e.g. light, temperature and humidity). Each coloured parameter represents a scale of values. Due to sample variability and visual reading, a sample with an analyte concentration close to the normal value gives a result of the higher value. Results usually always give a higher concentration of the true value. The detectable level of analytes present in the urine is given in the "Test Limits" section of each parameter; due to clinical variations in urine, lower concentrations may be detected.

12 I LITERATURE

1. GP16-A: Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline (1992); National Committee for Clinical Laboratory and Approved Standards (NCCLS).

SYMBOLS

	Attention, see instructions for use		Tests per kit	REF	Catalog number
IVD	For *in vitro* diagnostic use only	2°C–30°C	Store between 2-30°C		Do not reuse
	Manufacturer	LOT	Lot number		Expiry

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