UREA
BERTHELOT

INTENDED USE:

This reagent kit is intended for ***"in vitro"*** quantitative determination of Urea in serum, plasma and urine.

CLINICAL SIGNIFICANCE:

Urea is produced in the liver, as a waste product in the urea cycle from the catabolism of proteins in humans. Consequently, the circulation levels of urea depend upon protein intake, protein catabolism, and kidney function. Low levels of urea are not common. It can be seen in severe liver disease or malnutrition but is not used to diagnose or monitor these conditions. Low urea Level were also observed in normal pregnancy. Elevated levels of ureasuggest impaired kidney function but it may also be due to congestive heart failure, shock, recent heart attack or severe burns,bleeding from the gastrointestinal tract, and conditions that cause obstruction of urine flow or dehydration.

PRINCIPLE:

Urease catalyzes the conversion of urea into ammonia and CO_2. Ammonia released reacts with a salycilate in the presence of a nitroprusside and hypochlorite, giving a blue-green colored Complex. The absorbance of the colored solution is directly proportional to the urea concentration, when measured at 600 nm.

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Salycilate} + \text{Hypochlorite} \xrightarrow[\text{Nitroprusside}]{\text{Sodium}} \text{Blue Green Color Complex}$$

REAGENT COMPOSITION:

Reagent 1: Enzyme Reagent
Reagent 2: Chromogen Reagent
Urea Standard: 40 mg/dl.

SAMPLES:

Serum, EDTA or heparinized plasma, free of hemolysis. Urine diluted 1:99 in normal saline (multiply the result by 100).

WORKING REAGENT PREPARATION & STABILITY:

Step 1: Bring all the reagents to room temperature.
Step 2: Working reagent 1: Dissolve the enzyme reagent 1 in **deionized water with the volume indicated on the vial label. Working reagent 1 is stable for 75 days when stored at 2° to 8°C away from light.**
Step 3: Working Reagent 2: Reagent 2 (Chromogen reagent) is ready for use. Once opened stable for 6 months when stored at 2º to 8°C away from light.
Store the unopened kit at 2° to 8°C away from light.

GENERAL SYSTEM PARAMETERS:

Reaction type	End Point
Wave length	600 nm (580-630 nm)
Light Path	1 Cm
Reaction Temperature	37°C
Blank / Zero Setting	Reagent
Working Reagent 1	1 ml
Working Reagent 2 (Chromogen Reagent)	1 ml
Sample Volume	10 µl
Standard Concentration	40 mg/dl
Low Normal at 37°C	11 mg/dl
High Normal at 37°C	44 mg/dl
Linearity	200 mg/dl

ASSAY PROCEDURE:

	Blank	Standard	Sample
Working Reagent 1	1ml	1ml	1ml
Standard		10 µl	
Sample			10 µl

Mix and incubate for 5 Minutes at 37°C and then add

Working Reagent 2	1ml	1ml	1ml

Mix and after 5 minutes incubation at 37°C, measure the absorbance of standard and sample against the reagent blank at 600 nm (580-630 nm) within 60 minutes.

CALCULATION:

$$\text{Urea (mg/dl)} = \frac{\text{O.D Sample}}{\text{O.D of Standard}} \times \text{Conc. of Standard}$$

LINEARITY:

Reagent is Linear up to 200 mg/dl.
Dilute the sample appropriately and re-assay if urea Concentration exceeds 200 mg/dl.

REFERENCE NORMAL VALUE:

Serum/Plasma : 11-44 mg/dl
Urine : 15-30/24hrs
It is recommended that each laboratory should assign its own normal range.

QUALITY CONTROL:

For accuracy, it is necessary to run known controls with every assay.

LIMITATION & PRECAUTIONS:

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperatures as it may affect the performance of the kit.
3. Before the assay bring all the reagents to room temperature.
4. Avoid contamination of the reagent during the assay process.
5. Use clean glassware free from dust or debris.

BIBLIOGRAPHY:

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