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Measurement Technology Used in the Fully Automated Urine Chemistry Analyzer UC-3500

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The UC-3500 Fully automated Urine Chemistry Analyzer (Sysmex Corporation, Kobe, Japan) is a device that can carry out all operations from sampling to measurement in a fully automated manner. The company uniquely developed the technology for analyzing the colors on a urine test strip using a color CMOS sensor, which is the heart of the test strip measurement, to achieve high measurement accuracy. The colors on the urine test strip change as a result of a chemical reaction. The analyzer detects the change in color and outputs the result of measurement. In this method, light plays an important role in the analysis.

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How is light perceived by humans?

Most of the spectrum of visible sunlight is in the wavelength range of about 400 to 800 nm. The human eye is set to perceive this wavelength range as visible light and to sense the color.

The human retina has three types of photoreceptor cells (cone cells) which independently react to different wavelengths. These are designated as L cones (red cones), M cones (green cones) and S cones (blue cones). These three types of cone cells are in the retina and can sense red (around 610 nm), green (around 550 nm) and blue (around 450 nm) light. We can therefore say that these three colors, red, green and blue (R, G and B) are the colors that humans can perceive, and these are called the three primary colors of light. Yellow light is produced when red and green light are mixed. Likewise green and blue give cyan, and blue and red give magenta. Mixing of all the primary colors, red, green and blue, gives a white color.

In this manner, almost all shades can be created by mixing different proportions of R, G and B. Humans can perceive a wide range of colors created by combining these three primary colors.[1)]

For example, when white light, which contains R, G and B, is shone on an apple, G and B are absorbed and only R is reflected back. This makes us perceive the apple as having red color (***Fig. 1***). The measurement with the urine test strip exploits this phenomenon. It is possible to analyze the color of a pad on the test strip by measuring the intensity of R, G and B, using the same principle as used by the human eye. The UC-3500 uses a color CMOS sensor in the unit that detects reflected light.

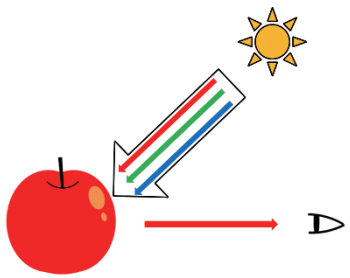

Fig. 1 *The mechanism that makes an apple look red*

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1. The mechanism of color using a CMOS sensor

The basic structure of the imaging unit (CMOS sensor) [2)]

Generally, an imaging system has two parts, a camera unit and a lens unit. ***Fig. 2*** shows the most basic configuration. Even in a relatively simple configuration, normally there are multiple numbers of lenses and diaphragms. Also the camera unit has an electrical circuit that controls the camera, corrects the image and outputs it. In many cases it has an anti-reflection (AR) filter also.

The lens unit gathers light coming from the object and forms an image on the imaging surface of the sensor.

Basic functions of the image sensor

The image sensor is a device that detects light, converts it into electrical charge, and processes it as an electrical signal (***Fig. 3***). The incident light is converted into an electrical charge by a photodiode, which is amplified and processed as an electrical signal. A photodiode can sense only the intensity of light, not its color. Therefore, color filters are used to pass only red, green or blue light and their intensity information is converted into color data (***Fig. 4***).

The image obtained by the image sensor consists of a collection of small squares. Each small square is called a picture element or pixel. Each pixel has the three color elements, red, green and blue, and 256 levels of light. The image is formed as a composite of these pixels (***Fig. 5***). The image sensor plays the role of determining each element value, red, green and blue, of the pixels.

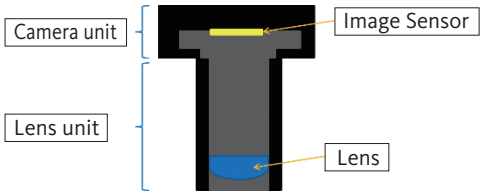

Fig. 2 *Structure of the imaging unit*

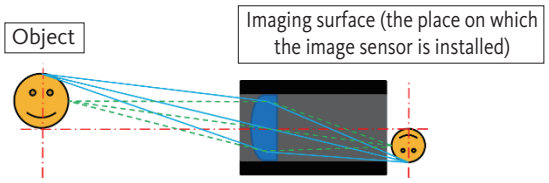

Fig. 3 *Functions of the lens unit*

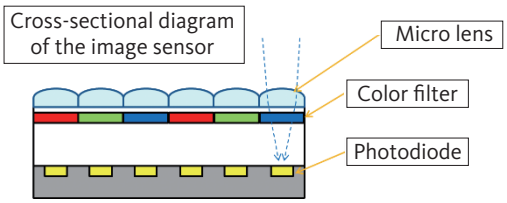

Fig. 4 *A general outline of the image sensor*

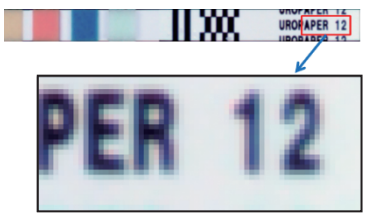

Fig. 5 *Image obtained by the image sensor. Each small square is a pixel.*

Each pixel of an image is composed of the three color elements, red, green and blue. In the image sensor, on the other hand, each pixel can receive only one of the three elements. To overcome this difference, the image sensor generates an image by complementing colors between adjacent pixels of sensor so that each pixel of image would have all the three elements, red, green and blue (***Fig. 6***). In the UC-3500 the test strip is imaged as shown in ***Fig. 7***.

2. Measurement technology of the UC-3500 which uses a color CMOS sensor

Photometric principle and useful features of urine test strip measurements

The UC-3500 photometrically scans the test strip using a color CMOS sensor and obtains two-dimensional image data of the entire strip. This two-dimensional image data is separated into the three primary colors of light and the reflectance value of each pad is obtained from that data by comparing the pad with the white base film of the strip. This reflectance value is then converted into the measured value using a calibration line that represents the chromogenic characteristics of each pad (***Fig. 8***).

Although there are some exceptions, color development on the test strip is designed so that the color becomes deeper as the reaction advances. The reflectance, which changes depending on the color intensity on the test strip, is used for measurement.

A highly durable white LED is used as the light source. In order to improve the measurement accuracy using the UC-3500, a correction for illumination intensity is completed every

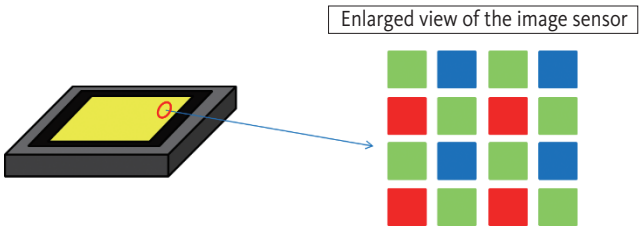

Fig. 6 *Structure of the image sensor*

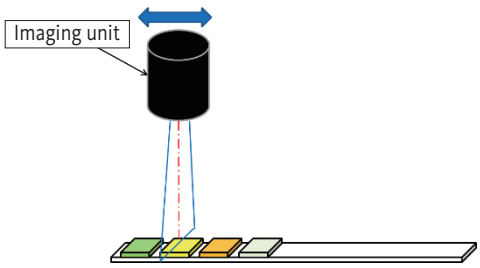

Fig. 7 *In the UC-3500, the optical unit, where the imaging system and illumination system are integrated, scans the test strip like a flat head scanner.*

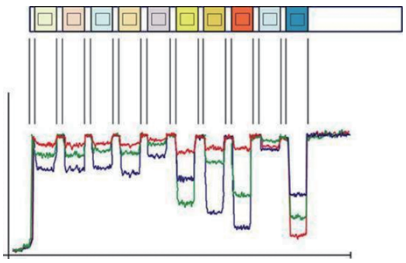

Fig. 8 *A graphic representation of image analysis by the color CMOS sensor*
The base film of the test strip is white and reflects light. This makes it possible to specify the size of each pad and its location.

time a test strip is scanned. The reflectance of an internal standard is used in this correction, as light reflected from the test strip surface is compared to the internal standard to determine the extent of its reduction. This gives the reflectance, which is converted into the measured value. This makes it possible to take measurements that are free from variations due to the test strip itself, the measuring environment, or the illumination conditions, etc., even without using a check strip or other quality measure.

Urine has different colors depending on the condition of the patient. It may sometimes be a "colored urine" *. Depending on the urine color it may affect the reflectance of the pads. To minimize this effect, a blank pad is also provided on the dedicated test strips (MEDITAPE UC) of the UC-3500 for correcting the effect of sample color on each test pad. This feature makes highly accurate measurements possible even with urine that is colored.

* Colored urine and false reaction of the test strip
The urine test strip is directly affected by urine color. We may get a false positive result especially when the urine color is similar to the positive color for a certain parameter.
In most cases, such effects of colored urine may be categorized as false positive reactions of the test strip. Among them are cases where a metabolic product of a drug colors the urine. In some instances this can be because of a metabolic product of a drug reacting with a reagent component in the test strip, affecting the color development on the test strip. This kind of false reaction can be caused by a reagent component of the test strip reaction system reacting with a drug metabolic product of a drug taken by the patient or a test strip buffered to be strongly acidic or strongly alkaline changing the pH of the sample and modifying a drug in the urine, impacting the result.

For example, patients under treatment with etodolac produce pale yellow urine. When phenolic metabolites of this drug react with diazo compounds of the bilirubin test pad, it assumes a pink to reddish brown color which may be wrongly assessed as a positive result.[3] This is an example of a case when a reagent component is affected. With epalrestat, the urine color becomes dark yellow to orange yellow, but it assumes a reddish brown color under alkaline conditions.[4] With the ketone body test pad for which the optimum alkaline pH is maintained, a reddish brown color develops. This is an example of a drug changing color development due to a change in pH. Thus, urine test strips have some limitations due to their inherent characteristics and they should be used after a thorough understanding of the effects of colored urine and drugs taken by the patient.

Advantages of image processing systems that use color CMOS sensors

1. Detection of pad location on the strip and site designation

By scanning the strip, the system can detect and display an error message when a wrong strip has been used, when the test pad on the test strip is displaced or peeled off for some reason, or when a viscous sample has covered more than one pad.

Causes of error
- Inappropriate positioning of the test strip
- Insufficient amount of light during photometry
- Abnormal distance between pads on the test strip
- Pad missing on the test strip
- Use of a test strip different from the designated one

As the locational information about the test pad can be accurately obtained during a measurement, the system can designate the site at which the color should be assessed, which enables more accurate analysis. This function helps prevent misjudgment arising from the "window frame phenomenon" **.

** Window frame phenomenon of urine test strips
Each test pad is a cuboid. Therefore, the urine seeps in through the side surfaces also, apart from the top surface. Thus, more than the specified amount is absorbed at the edges, which makes the shade darker there, and raises the possibility of improper measurement (***Fig. 9***). Therefore the color along the margin areas of the test pad is not used for the measurement.

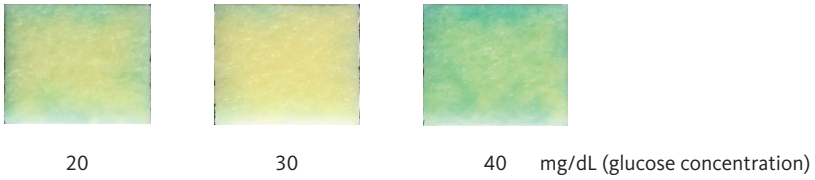

Fig. 9 *Window frame phenomenon in a glucose test pad*
The margins of the test pad have a darker shade compared to the central part.

2. Differentiation of hemoglobin from red blood cells

The occult blood test strip measures hemoglobin of red blood cell origin. When red blood cells are present in the urine and hemolysis advances resulting in release of hemoglobin, the occult blood test pad shows uniform color. If the red blood cells are still fresh and hemolysis occurs on the test pad, the color development would be non-uniform as the reaction occurs in a localized fashion (***Fig. 10***).

When the color development is localized, the UC-3500 tends to assess the reflectance as higher (lower color depth) than the actual level (***Fig. 11***). In the UC-3500 Series, the variation of reflectance on a pad is assessed to determine whether the color was caused by reaction with hemoglobin or by localized reactions involving hemolysis of red blood cells, and different calibration curves are used for these two cases. This enables more accurate measurement (***Fig. 12***).

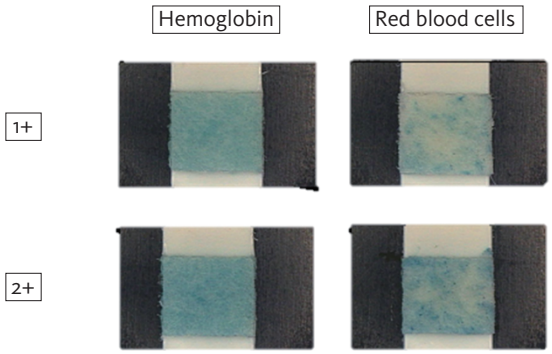

Fig. 10 *Examples of color development caused by hemoglobin and by red blood cells*

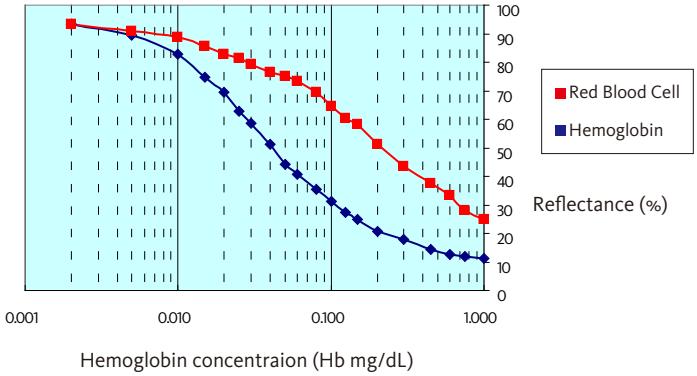

Fig. 11 *Difference in reflectance between hemoglobin and red blood cells*

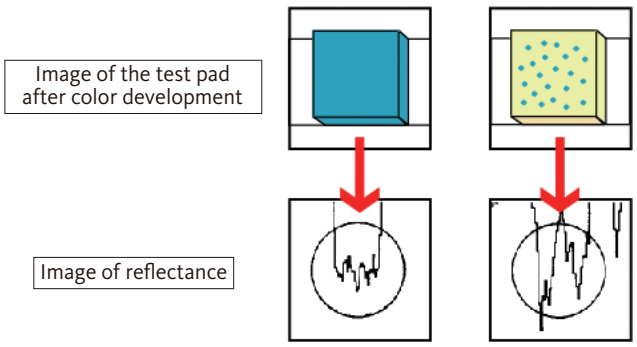

Fig. 12 *Difference in the variability of reflectance between hemoglobin and red blood cells*

3. Identification of abnormal color of the test pad

What we should keep in mind when assessing abnormal color development of urine test pads is whether the abnormality is due to a drug, etc. present in the urine sample, degradation of the test strip, or an insufficient amount of sample applied.

Abnormal color development in test strips is monitored on the UC-3500. The color of each pad is compared with normal color development for each parameter to detect any abnormal color development pattern, and if suspected the marks "?" and "!" are output to warn of possible abnormality.

Color measurement by the CMOS sensor can be used for detecting abnormal color development. Samples that had been measured using the analyzer and also assessed to have highly abnormal color through naked eye observation were found to have a proportion of R, G and B that was different from normal samples. This relationship is used by the technology for differentiating abnormal coloring from normal coloring.

When actually analyzing samples, RGB is further analyzed and two types of warning signs are output depending on the intensity of abnormal color development. However, if the color is only mildly abnormal, the differentiation may be difficult to detect because the difference in color is small and the result may be different from that of macroscopic measurement.

Principle of the specific gravity measurement

The UC-3500 uses the refractive index method for measuring specific gravity (***Fig. 13***).
Light emitted from the LED source passes through a prism and gets reflected from the surface where it comes into contact with the sample and enters the detector.[5,6]

Here the refractive index differs depending on the specific gravity of the sample dispensed into the flow cell, and the position of the light incident on the detector changes according to the specific gravity. The refractive index of the sample is obtained from the position of this incident light and converted into specific gravity.

Random reflection occurs when light is passed through a cloudy sample and accurate measurement of specific gravity may become impossible. Reflection refractometry measures the refractive index without passing the light through cloudy urine. Therefore the effect of random reflection is avoided, making more accurate measurement possible.

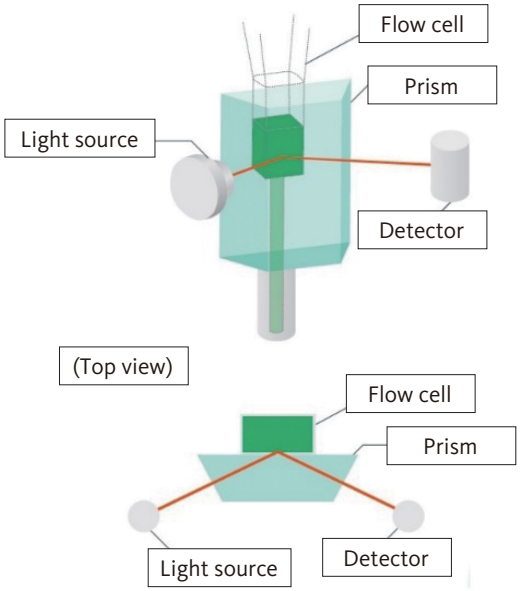

Fig. 13 *The UC-3500 flow cell (Reflection refractometry)*

Specific gravity markings are etched on the refractometer used for measuring urine specific gravity. These are based on the correlation of urine refractive index and the measured reference interval of urine specific gravity of a large number of healthy individuals. These markings are harmonized with the standards of the JSCP nomogram recommended in 1979 by the Standardization Committee of the Japan Society of Clinical Pathology.

To convert refractive index to specific gravity, the refractometer of the UC-3500 uses specific gravity calibration solutions which have known values based on measurement by a standardized "JSCP nomogram-based refractometer" ***.

*** JSCP nomogram-based refractometer
It had been reported at the International Symposium on Quality Control held in Tokyo in 1974 that the urine specific gravity in Japan differed from that measured by College of American Pathologists (CAP) by 0.003 to 0.005 in the specific gravity range of 1.010 to 1.030.

In 1979, the Standardization Committee of the Japan Society of Clinical Pathology prepared a JSCP nomogram and the scale markings were standardized. The UC-3500 uses a refractometer based on this nomogram. The differences in urine specific gravity measured by refractometers of different manufacturers were eliminated in this manner, improving the accuracy of measurement. Nevertheless in countries where the dietary habits and environment are different from Japan, the composition of urine would be significantly different. Therefore, the values measured by urine refractometers prepared in Japan may not always match with the nomograms in other countries.

The Standardization Committee of JSCP has stated that urine contains much more urea and common salt than other components and these impact urine specific gravity measurements made using the refractive index method and the weighing method. The urine of residents in the US has a high urea content whereas the salt content is higher in the urine of Japanese. Due to these differences the urine specific gravity measured by the refractive index and weighing method differ between Americans and Japanese, and the difference between nomograms prepared in the two countries arises from this background.[7)]

Principle of measuring urine color and cloudiness

There are 4-color LEDs that are used as the light source. Absorbance data is obtained through colorimetric measurement in a flow cell. This absorbance data is classified into five ranks for each of four wavelength ranges and recognized as the “color tone rank”. The urine color (seven types, namely L-YELLOW, STRAW, AMBER, YELLOW, RED, DK-BROWN, OTHER) is output on the basis of the color tone rank thus recognized.
Cloudiness correction is done for classification and recognition of urine color.

Precise dispensing of sample on to the test strip in the UC-3500

To improve the accuracy of measurements made with the UC-3500, the sample is accurately added drop-wise on each pad of the test strip so that the optimum amount of sample is received by the pad.
The position of the test strip is measured by a built-in sensor and the sample is loaded drop-wise on each pad to match the designated position of measurement. The sample is dropped from a certain height from the surface, taking into account the thickness of the pad and the position of the strip.
Such drop-wise addition facilitates the addition of a stable amount of sample to the pad and prevents mutual contamination of reagents, which contribute to making the test strip reaction more accurate.

Quality control and mode of measurement

The UC-3500 has three measurement modes, namely, [C.] series, [NO.] series and [#.] series. The [C.] series can output the reflectance for each parameter. On the UC-3500, quality control is done through the use of qualitative values. However, depending on the combination of the UC control lot and the test strip lot, different measurements can yield different judgement values. In such cases, by checking the reflectance values, we can find out whether such variation is because the reactivity is around the cut-off level (border) between the two adjacent qualitative judgement values or some other reason.

The [NO.] series is the mode used for routine testing. If a sample is to be analyzed out of turn, i.e. a STAT or emergency sample, we can easily verify the sample that was measured out of turn by switching over to the [#.] series mode before taking the measurement.
The data output depends on the mode. For instance, the [C.] series outputs qualitative values and reflectance. This mode does not output abnormality warning flags, nor does it do negative correction. On the other hand the [NO.] series and [#.] series output the qualitative and semi-quantitative values.

The UC-3500 is a device that can register quality control bar codes. When analyzing samples with a quality control barcode attached to it, it automatically outputs in the [C.] series mode. This feature eliminates the risk of results of quality control samples not being output in the [C.] series or the results being output in some other series because someone forgot to switch to [NO.] series or the [#.] series after measuring the sample.

3. Urine test strip measuring technology of the UC-3500

Avoidance of false negative reactions on the test strip

1. Avoidance of the effect of ascorbic acid

Currently, a variety of products, such as food and drinks

(soft drinks, fruits, food additives, etc.) and pharmaceutical products (health drinks, tablets, infusion fluids, etc.) contain considerable amounts of ascorbic acid (AsA: vitamin C). People who go for urine testing may have consumed large amounts of ascorbic acid through such sources. It is widely known that AsA ingested in this manner affects urine test results as most of it is excreted into the urine. The excretion of AsA into urine generally peaks about 4 to 6 hours after oral ingestion. If 1,000 mg is taken orally, 100 mg/dL or more can be excreted in the urine. Gradually the rate gets reduced and almost all of it is excreted within about 24 hours.[8]

The occult blood test pad is affected by AsA because AsA is a stronger reducing agent than chromogens such as tetramethylbenzidine because of how it captures active oxygen [o] earlier than the chromogen, yielding a false negative result (***Fig. 14***). To avoid this, an oxidizing agent like iodic acid is added to the test strip. This oxidizing agent oxidizes AsA before it captures active oxygen. This prevents the effect of AsA (***Fig. 15***). With this method, the result does not become negative even if the AsA concentration in the sample is as high as 100 mg/dL. We can thus assume that the effect of AsA can be minimized by adopting this measure. Unfortunately, we cannot completely prevent false negative results as there are many other reducing substances in urine.[9]

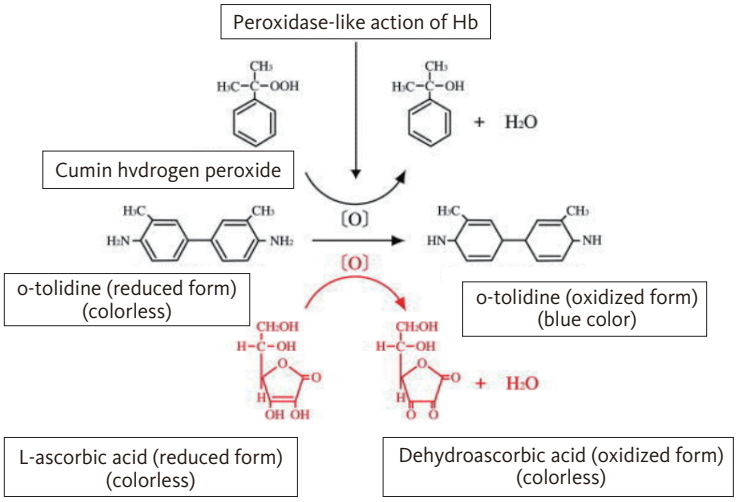

Fig. 14 *Mechanism of inhibition of the reaction by ascorbic acid*

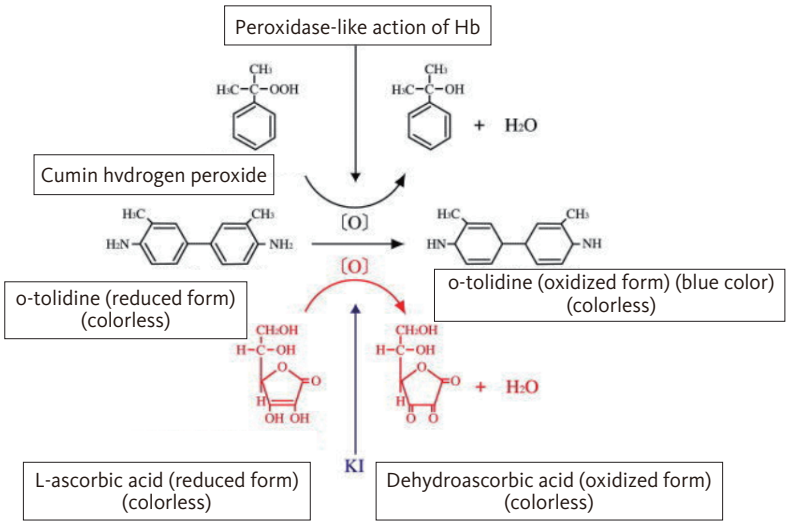

Fig. 15 *Prevention of inhibition of the reaction by ascorbic acid*

2. Avoidance of false positive results from the bilirubin test pad caused by drugs (negative correction of bilirubin result)

The urine of an individual taking etodolac reacts with the bilirubin test pad giving rise to a red color, for instance. This abnormal color is very similar to that which presents in the urine of a person with bilirubinuria and it is impossible to detect this false reaction with routine devices.

However, because of the special method of analysis of the reagent strips used by the CMOS sensor in the UC-3500, this series is now capable of identifying true bilirubinuria. When the system judges that abnormal color development is particularly high, it corrects the result to the negative range (negative correction). The system affixes a "?" flag along with the "(-)" result to distinguish the samples subjected to such negative correction from ordinary negative samples.

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