Urine Specific Gravity: The First and the Latest

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FIRST MEASUREMENT

Near the start of the seventeenth century, a young physician found himself in quite an awkward situation. He was a visitor in London from the low countries and found himself in conversation with a group of noblewomen, including at times the queen, from about three o'clock in the afternoon until three o'clock in the morning. All this time he felt it would have been impolite to excuse himself to take care of bodily functions.

When he was finally able to leave, despite his discomfort, he used the opportunity to carry out an experiment. He urinated through a napkin, and was fascinated to discover that there was no "sand" in the urine. Current Galenic doctrine said that urine held long in the bladder would become concocted and concentrated enough to produce bladder stones, a common problem of the time (now known to be diet-related). The young physician, Joan Baptista van Helmont, concluded he had better think for himself rather than be guided by medical literature of the time (1). Through efforts such as this impromptu experiment, he became known as one of the original thinkers in medicine of his time.

A hallmark of the success of the Diagnostic Division of Miles Inc. in the field of urinalysis has been an enthusiasm like that of Van Helmont for stepping beyond the commonly accepted vision of the time. This began in 1941 with CLINITEST® reagent tablets and continued in 1956 with CLINISTIX® reagent strips for glucose in urine. Breakthroughs continued with the first multiple reagent strips in 1958, the first urinalysis automation in 1971 and the first reagent strip for urine specific gravity in 1981 (2). In 1994, it has meant introduction of the CLINITEK® ATLAS™ Urine Chemistry Analyzer, which includes a new concept for measurement of urine specific gravity.

Van Helmont was the first to urge the use of specific gravity as part of a true urine analysis (3). For his measurements he weighed a volume of rain water, weighed the same volume of urine, and then divided the weight of the urine by the weight of the rain water. This was accurate, but very tedious. Moreover, given the less advanced understanding of physiology of his time, he had no basis for using any of the infomation he generated. For example, although he spent much time investigating bladder and kidney stones, as well as much time with measurements of urine specific gravity, he never made the connection that persons who keep their urine specific gravity low by drinking lots of liquids are much less likely to get stones. Four centuries ago, Van Helmont was just beginning to touch on the utilization of measurements of urine specific gravity in medicine, and never did find a use for it that we would regard today as appropriate.

THE IMPORTANCE OF URINE SPECIFIC GRAVITY

Now there is a good understanding of the ways in which measurements of urine concentration may be utilized. In some cases, a very accurate measurement of the concentration is important. For example, in healthy young adults, the urine specific gravity may range from 1.002 to about 1.035. If the urine remains fixed at a specific gravity of 1.010, even with low fluid intake, this is a sign of kidney failure: the tubules are failing to reabsorb liquid. With restricted fluid intake, the kidneys should produce urine with a specific gravity above 1.025.

More commonly, an approximate value of the specific gravity is adequate, since knowledge of the concentration of the many chemical constituents in urine helps in the interpretation of other results in the standard urinalysis. For example, a small concentration of protein in a very dilute urine is of greater significance than a small concentration of protein in a very concentrated urine.

FIRST IN EASE OF USE

Reagent Strip Revolution in the Urinalysis Laboratory

MULTISTIX® reagent strips have become such a routine part of the functioning of the medical laboratory of the 1990s that it is easy to forget the revolution in urinalysis begun by Miles fifty years ago, with the introduction of tablet and strip tests. To analyze for substances dissolved in urine the laboratorian now requires only a reagent strip, a color chart and a couple of minutes to carry out an analysis that earlier would have required a significant amount of laboratory equipment and many hours of work. By the 1970s, many reagent strip tests had been introduced by Miles:

- glucose, an indicator of carbohydrate metabolism defects;
- acetoacetic acid, an indicator of abnormalities of carbohydrate or lipid metabolism;
- protein, an indicator of kidney damage;

- blood, an indicator of kidney damage, infection, or cancer;
- nitrite, an indicator of infection;
- bilirubin and urobilinogen, indicators of liver damage; and
- pH, a measurement often helpful in connection with some of the other tests.

Of the group of chemical and physical tests that are generally part of a routine urinalysis, only the measurement of urine specific gravity had resisted efforts to adapt it to a reagent strip.

Many laboratories made specific gravity measurements with a urinometer, a hydrometer calibrated for use with urine. It requires a relatively large amount of urine and operates based on Archimedes' Principle. Other laboratories used a refractometer, also calibrated for use with urine. Although an indirect measurement of specific gravity, relying on a normally present mixture of urea and salts to provide the appropriate correlation, the refractometer provides quite a good determination of specific gravity and requires only one drop of urine for the standard manual device, a Total Solids (TS) Meter. Nevertheless, this one determination does require extra time to add sample, view the result, and clean the device. It was clear by the early 1970s that a dry reagent strip test for specific gravity would be welcomed by laboratories interested in reducing urinalysis workloads.

Early Attempts at a Specific Gravity Strip Test

Scientists at Miles tried several approaches for the development of a specific gravity strip test. In one approach (4), the sensitivity of reactions to concentrations or dissolved solids was utilized. For example, a reagent strip was prepared which contained an enzyme, its substrate, and a color generation system. When the strip was dipped in urine of low specific gravity, a large amount of color was generated, whereas in a urine of high specific gravity, much less color was developed. Unfortunately, interfering substances present in some urines, together with a marked temperature effect on the reactions, combined to make this approach impractical.

An approach carried much further into development used nylon microcapsules containing one half of a color generating coupling indicator system (5). These capsules would burst from the osmotic pressure differential in low specific gravity urines, but high specific gravity urines were approximately isosmotic with the capsules, and thus would develop little color. Unfortunately, in this approach the yield in the preparation of the microcapsules was rather low, and the color differentiation was not outstanding. The approach was abandoned.

Success at a Specific Gravity Strip Test

Persistence and creativity eventually provided a successful approach (6). The test is based on the properties of a polyelectrolyte, in this case poly(methylvinyl ether/maleic anhydride), which is hydrolyzed during the manufacture of the reagent to produce carboxylic acid groups, as shown in Figure 1.

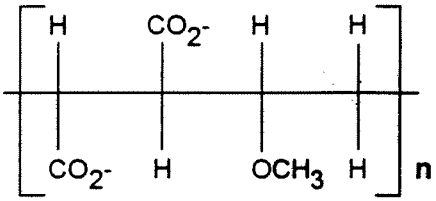

Figure 1. Structure of the polyelectrolyte in the urine specific gravity reagent strip, poly(methylvinyl ether/maleic acid).

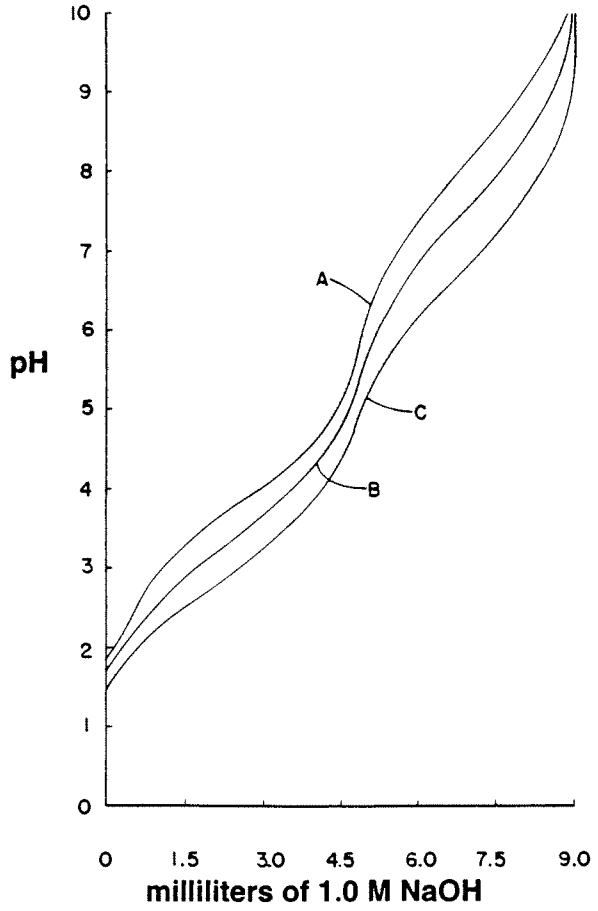

Figure 2. Titration curve, poly(methyvinyl ether/maleic acid). A. No added salt. B. Titration in O.1 M NaCl. C. Titration in 1.0 M NaCl.

In a polyacid, each carboxylic group is not free to dissociate independently, as would be the case (approximately) for an equivalent number of acetic acid molecules. Rather, the tendency of each group to dissociate depends on whether neighboring groups have already dissociated. Thus, in the titration of a polyacid, one does not obtain a sharp inflection point, as for a simple weak acid. Rather, one obtains a curve such as the top curve (A) in Figure 2.

An additional characteristic of a weak polyacid is that the ease of ionization is a function not only of the ionic state of neighboring groups, but also of the number of neutral salt ions in the vicinity. A "cloud" of counterions tends to shield the charge of each ionized carboxylic acid group from the nearby carboxylic acid groups. This allows these groups in turn to behave more independently of each other, and to become more easily dissociated. This effect is shown in Figure 2. At increasing salt concentrations, the same amount of added sodium hydroxide produces lower pH readings (B,C).

The reagent strip is formulated by titrating hydrolyzed polymer to a pH near 7.75, and adding a pH indicator, bromothymol blue. The solution is impregnated into filter paper, dried and attached to a plastic handle in the same manner as the other MULTISTIX® 10 SG urine reagent pads. When the specific gravity pad is dipped into a low specific gravity urine, the pH remains high, with a dark blue-green color produced by the ionized indicator. In a high specific gravity urine, containing high concentrations of ions, the screening effect produces more dissociation in the polyacid, a lower pH, and the yellow (protonated) form of the indicator. Seven color blocks cover the customary range of urine specific gravity, from blue at 1.000 to yellow at 1.030, in increments of 0.005.

As might be expected, the pH and buffering capacity of the urine can affect the results. A correction factor

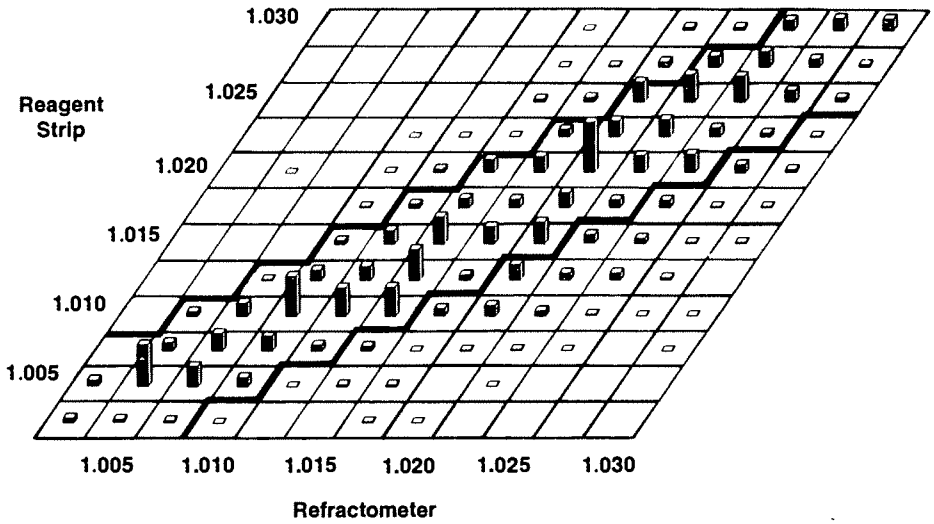

Figure 3. Correlation of Specific Gravity reagent strip results with refractometric determination of specific gravity. The height of the block at each value represents the number of results at that specific gravity. The pH correction of +0.005 for samples with pH 6.5 or greater has been applied. The heavy lines represent the agreement within 1 color block.

of + 0.005 is recommended with urines having a pH greater than 6.5 for customers reading the strip visually with the color chart. If the customer uses instrumental reading of the strip, the instrument makes the correction automatically.

In a comparison with the refractometric method, the reagent strip produced good results, consistent with the requirements for routine urinalysis. These results are shown in Figure 3. Overall, 84% of all results were within 0.005 of the refractometer results. In a similar comparison with the urinometer, 89% of the results fell within +/- 0.005 of the comparison method (7).

THE FIRST AUTOMATION FOR URINALYSIS

Before research had begun for the specific gravity reagent strip, there was a recognition that high volume laboratories needed a fully automated urinalysis system. The CLINILAB® Automated Urine Analyzer, introduced in 1971, was designed to dispense reagent pads from cassettes, and to apply the urine samples by pipet to each pad (8).

To accompany the reagents, an automated specific gravity module was designed (9). The module, illustrated in Figure 4, measured the time required for a precisely measured drop of urine to fall a distance between two points in a temperature-controlled column of water-immiscible fluid of slightly lower density

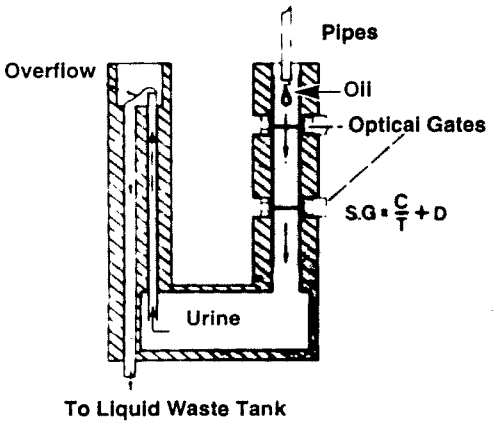

Figure 4. Diagram illustrating the operation of the specific gravity column. The urine sample is pipetted into the column of oil. The drop is timed between the two optical gates. Excess urine overflows into a waste tank. Reprinted from *Clinical Chemistry*: 1972; 18:Figure 6:789-793, courtesy of the American Association for Clinical Chemistry, Inc.

than the urine. The system was calibrated with distilled water and a saline solution at 1.040 g/cc. After the falling times for distilled water (T_1) and the 1.040 standard (T_2) were determined, the column constant (C) was calculated

$$C = \frac{40 \times T_1 \times T_2}{T_2 - T_1}$$

as was the density of the column fluid (D)

$$D = \frac{40 \times T_2}{T_2 - T_1}$$

For subsequent urine samples, the specific gravity could be calculated by

$$\text{Specific Gravity} = \frac{C}{T_s} + D$$

where T_s is the falling time of the sample drop of urine.

In a comparison study of the CLINILAB® method and the refractometric method, results generally agreed within +/- 0.005, with better agreement at lower specific gravities. Some results are shown in Figure 5 (8).

The performance of the specific gravity column served customers well for about two decades, and was incorporated, with some improvements for reliability, into the next generation automated instrument, the CLINITEK® Auto 2000, introduced in 1984. Still, in the design of the newest automated system, the CLINITEK® ATLAS™ Urine Chemistry Analyzer, there was an opportunity to utilize a different concept, one which held the prospect of further increasing reliability through simpler design.

THE LATEST AUTOMATION IN URINALYSIS

The CLINITEK® ATLAS™ Urine Chemistry Analyzer

The CLINITEK® ATLAS™ Urine Chemistry Analyzer is a fully automated reflectance spectrophotometer which uses a pipet to apply urine samples precisely from a carousel containing approximately 40 urine specimens onto a reagent roll capable of 490 routine urinalysis profiles. Through dry reagent strip technology, results are provided automatically for glucose, bilirubin, acetoacetic acid, occult blood, pH, protein, urobilinogen, nitrite, leukocytes and urine color. Automated determination of specific gravity is also a key feature.

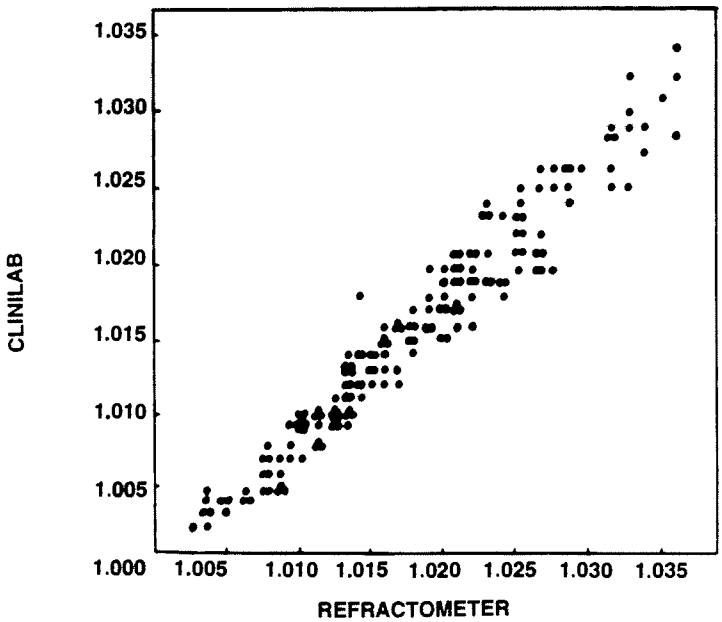

Figure 5. Falling drop specific gravity column results vs. refractometer, n=170. Reprinted from *Clinical Chemistry*. 1972; 18:Figure 1:794-799, courtesy of the American Association for Clinical Chemistry, Inc.

The New Design

The need for high speed, together with good accuracy for specific gravity in an automated urinalysis instrument, led to a consideration of methods based on refractive index. Measurements with commercial refractometers have shown that urine refractive index is highly correlated with urine specific gravity. Moreover, the response of an optically based refractometric sensor is immediate.

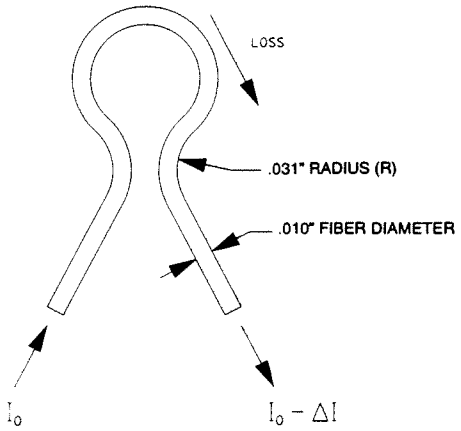

Figure 6. Shape of the omega fiber used in urine specific gravity determination. The light enters one side and exits on the other. The loss of light within the fiber (ΔI) is highly correlated with urine specific gravity.

In a cooperative effort with Battelle Memorial Institute, a fiber optic-based urine specific gravity sensor was developed (10). A drawing of the active portion of the sensor is shown on Figure 6. This omega shaped loop is formed at the middle of a 44 inch long plastic optical fiber with a diameter of 0.010 inches.

The omega loop is fixed at the bottom of a well that has a diameter of 0.140 inches. One end of the optical fiber is connected to a light source and the other end is connected to a light

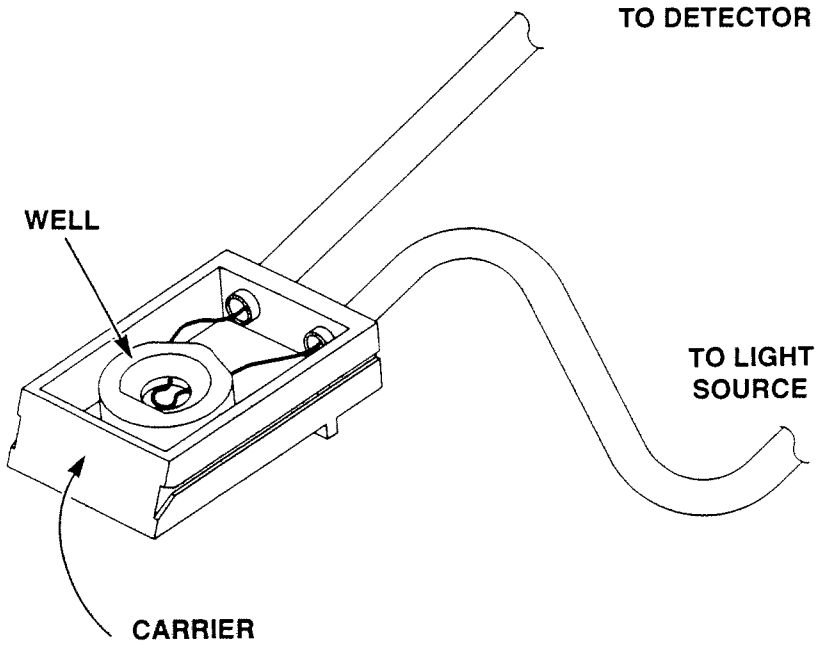

Figure 7. Cut-away view of the SG well. The omega-shaped loop is placed at the bottom of a well that is inside a carrier. One fiber goes to a light source, and the other to a detector. The cavity surrounding the well is backfilled with epoxy. Not shown is an upper section that keeps fluid in the well after the device is tilted 45 degrees.

detector. A cut-away drawing of a simplified SG sensor is shown in Figure 7. The cavity surrounding the well is back-filled with black epoxy, and a lip is added to the center opening to help retain fluid in the well after the device is mounted at 45 degrees.

In operation, the transmission of the fiber is measured first with a reference solution, such as distilled water, in the well. Then a urine sample is dispensed into the well, displacing the reference solution, and the transmission is measured again. From the ratio of these two signals, the urine specific gravity is determined.

The Physics

The top of Figure 8 shows what happens to light when it goes through the side of a fiber optic immersed in urine. Except for a very small amount of reflection, most of the light passes through the fiber optic as it would pass through an ordinary window pane. Thus, one can see through the fiber from the side.

The bottom of Figure 8 shows what happens when light traveling

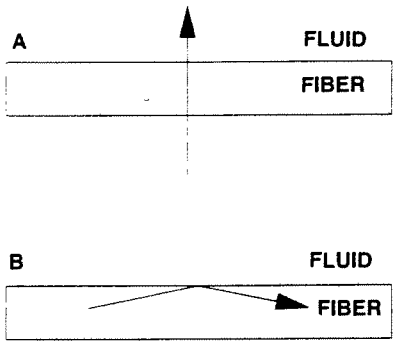

Figure 8. In the top drawing (A), light approaching the interface of the fiber optic and fluid (urine) passes through when the angle is close to the perpendicular. In the bottom drawing (B), light is 100% internally reflected when approaching the interface at a near grazing angle. The angle at which light starts to be totally internally reflected is called the critical angle.

along the inside of a fiber optic hits the same boundary. In this case 100% of the light is reflected back into the fiber. This is why light can travel miles through a fiber optic even though there are millions of reflections before the light finally leaves the end of the fiber.

Both of these are examples of the same optical law: when light is in a medium with a high index of refraction (fiber optic) and approaches the boundary with a medium of lower index of refraction (urine) at an angle greater than the critical angle, the light is totally reflected. In the top of Figure 8, the light is traveling perpendicular to the fiber/fluid boundary. Zero degrees is less than any finite critical angle and the light passes through. In the bottom of Figure 8, the light is traveling almost parallel to the boundary. Close to 90 degrees, this light is approaching the boundary with an angle larger than the critical angle and is 100% reflected.

The critcal angle is given as the arcsin of the ratio of the lower index of refraction divided by the higher index of refraction. For a fiber optic with an index of refraction of 1.333 and a urine sample with an index of refraction of 1.480, the ratio is 0.901. The sine of 64.25 is 0.901, and therefore 64.25 is the critical angle for these conditions.

The omega loop is interesting because it is not at either of these extremes, but halfway between. Figure 9 shows a section of the sensor near the first bend of the loop. For a straight or slightly curved fiber, the light will always be totally internally reflected. At a very sharp bend, the light can escape. In fact, when vendors were approached to produce this device, they suggested that the omega loop be removed because too much light would be lost at the bends.

In the omega sensor, light is lost from rays such as Ray A in Figure 9. The light is approaching the boundary at close to the perpendicular, less than the critical angle. With this shape, there is always an open window allowing some of the light to escape from the fiber into the urine.

Ray B of Figure 9 is shown approaching the surface at an angle slightly larger than the critical angle and it is totally reflected. Those rays

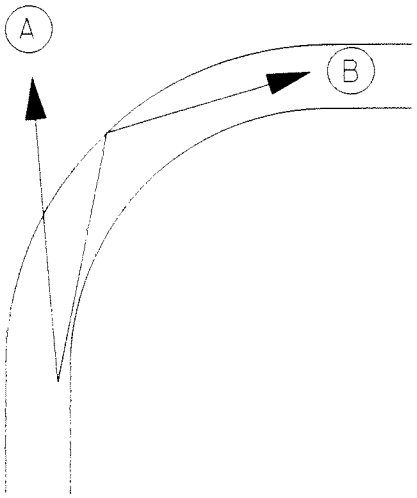

Figure 9. At the sharp bends of the omega loop, many of the light rays are near the critical angle. Ray A is shown less than the critical angle and the light passes through. Ray B is shown greater than the critical angle and light is internally reflected. If the specific gravity of the urine increases, the critical angle will increase and then both rays will pass through, leading to a loss of light from the fiber optic.

that are at the critical angle form the frame around the window. When a sample with a higher index of refraction is placed around the fiber, the critical angle becomes larger. More rays, including Ray B, have an angle less than the critical angle and more light passes through. Increasing the index of refraction of the urine effectively opens the window to let more light through.

The reference solution for the CLINITEK® ATLAS™ Urine Chemistry Analyzer has a specific gravity of 1.000 and an index of refraction of 1.333. A urine with a specific gravity of 1.060 (a value which can occur in humans through the presence of radiopaque dyes) will have an index of refraction of 1.353. The index of refraction of the plastic fiber is 1.48. Going from a very low to a very high specific gravity urine changes the critical angle from 64.25° to 66.09°. This slight increase in critical angle can result in well over 30% light loss from some fibers.

Critical Performance Factors

The angle at which the light approaches the surface of the fiber directly affects light loss. Hence anything that causes a change in shape in a particular omega loop will also cause a change in sensitivity and performance. For this reason, the omega loops are formed at a high temperature to insure a permanent set in shape.

The amount of light lost at the sharp bends of the fiber optic is a function of the index of refraction of the medium at the surface of the fiber. Hence any substance with a different index of refraction that coats the fiber will change the sensitivity of the sensor. For clinical samples, it was found that regular cleaning was required to keep the surface free of deposits.

Methods of cleaning were explored extensively during optimization of SG sensor performance. The use of a 10% bleach solution was found to improve performance if implemented every day or before every 500 specimens for large volume laboratories. Unfortunately, this cleaning alone was not always adequate. Depending on the types of specimens within a rack of samples, degradation of performance could occur before even a cleaning at the end of the rack could help. This led to more thorough rinsing between each sample.

As urines are tested on the CLINITEK® ATLAS™ Urine Chemistry Analyzer, a 300 μL aliquot of the specimens is dispensed into the SG well, containing the omega sensor. After the specific gravity of the sample is determined by the sensor, a specified amount of rinse solution is dispensed into the SG well. The rinsing of the SG well is done by displacing the urine sample with the rinse solution. Consequently, it is important that adequate rinse volume be used to remove all traces of the previous sample. This minimizes carryover of one sample to another and buildup of foreign material on the fiber optic sensor. A volume of 800 uL of the rinse solution was found to eliminate degradation of the SG well performance during routine testing. This, coupled with the 10% bleach cleaning, resulted in optimal performance of the SG sensor.

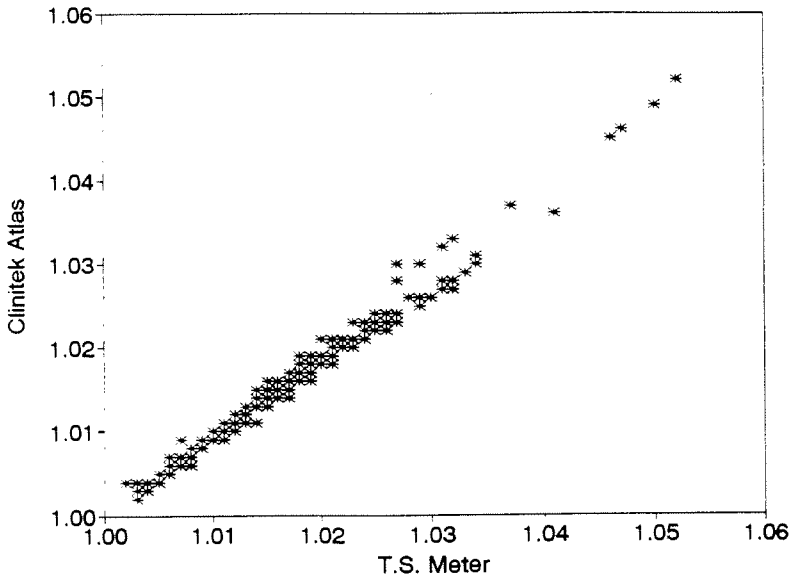

Figure 10. Specific Gravity performance on the CLINITEK® ATLAS™ Urine Chemistry Analyzer during an external evaluation at Miles Inc. with 209 clinical specimens. ($y=0.928x + 0.072$, $r^2 = 0.980$)

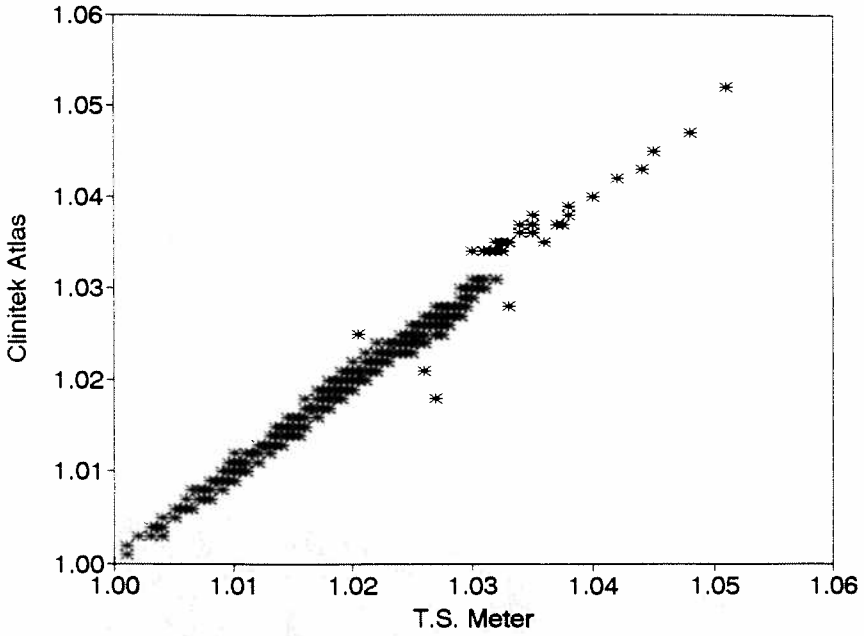

Figure 11. Specific Gravity performance on the CLINITEK® ATLAS™ Urine Chemistry Analyzer during an external evaluation at a large private clinical laboratory using 1112 clinical specimens. ($y=0.965x + 0.035$, $r^2=0.986$)

Aside from the 10% bleach daily cleaning, which is done automatically by the instrument once the user provides a tube of the solution, the SG well requires little maintenance. Calibration is only required every two weeks or before every 500 specimens. A three-point calibration is utilized in the algorithm to enhance performance throughout the SG range.

Clinical Performance

Two clinical studies, one internal and one external, showed the superior performance of this SG sensor. The internal study was done with clinical samples obtained from hospitals in the Elkhart/South Bend area. The samples were no more than 24 hours old and were refrigerated until analyzed. An aliquot of each sample was tested on two manual refractometers (T.S. Meters) by two readers. Another aliquot was tested on the CLINITEK® ATLAS™ Urine Chemistry Analyzer. The data in Figure 10 demonstrate the good agreement between the refractometer and the SG well. Of the 209 samples tested, most of the results (94.2%) were within 0.003 of the T.S. meter.

A CLINITEK® ATLAS™ Urine Chemistry Analyzer was taken to a large private clinical reference laboratory for the external evaluation. A total of 1112 clinical specimens were tested by Miles personnel over two days. These samples were tested using the T.S. Meter (2 readers, each using one refractometer) and the CLINITEK® ATLAS™ SG well. As shown in Figure 11, agreement between the SG well and the refractometer was excellent in this study. A majority of the results (98.7%) were within 0.002 of the T.S. meter results.

The creativity that went into this new system, as well as the quality of the results, serve as additional exampes of why Miles Inc., Diagnostics Division, continues to be the world leader in the field of urinalysis.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of Paul F. Corey with Figure 1; Alan E. Burkhardt with Figure 2; Eric Miller with Figures 3, 4 and 5; Cynthia S. Postma and William E. Bigler with Figure 7; and Jack L. Zuidema with Figures 6, 7, 8, and 9. We thank several persons for helpful comments on the manuscript, particularly Donald R. Parker, Mary Lou Gantzer, Richard J. Tyhach, L. Norman Gavazzoni, and Kerry J. Wilson.

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Jennifer Farr was born in State College, Pennsylvania and grew up in Northeastern Indiana. She obtained a B.S. in Chemistry from Purdue University in 1987. Prior to joining Miles in 1990, she was with Central Soya Inc. in Fort Wayne, Indiana working on lecithin product development for food and agricultural applications.

Since joining Miles, Jennifer has been involved with urinalysis reagent research and development. More recently she has been active in the development of the CLINITEK® ATLAS™ Urine Chemistry Analyzer and continues to be involved in future enhancements of the instrument.

Jennifer is an active member of the American Association for Clinical Chemistry and the American Chemical Society.

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Willis E. Howard, III, also known as Bill, was born in Oklahoma City in 1948, although much of his childhood was spent in Huntsville, Alabama. He obtained a B.S. from Samford University and then a Ph.D. from Northwestern University in 1976. Much of his thesis work in physical chemistry and laser spectroscopy was performed at the Technical University of Munich in Germany. Bill did postdoctoral work at the University of California in Irvine and came to Miles in 1979. At Miles he has worked in the areas of fluorescence and reflectance spectroscopy, algorithm development, software validation and optical engineering. He has worked on products including the CLINITEK® 200+ and CLINITEK® ATLAS™ Urine Chemistry Analyzers. He was Chairman of the Miles Science Forum in 1990. In his free time, Bill can be found reading, listening to music, eating Chinese food, enjoying his home computer, or racing his boat up and down the river.

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Bill received a bachelor's degree in Chemistry from Harvard College and a Ph.D. from Cornell University. After postdoctoral work at Washington State University, he joined Miles in 1975. Although he has had brief sojourns in Marketing, Manufacturing and Quality Assurance, he has spent most of his time in R&D, split between Urinalysis and Diabetes. Aside from a two year (1985-87) assignment in Leverkusen, Germany, all the intervening years have been spent in northern Indiana.

Currently, he manages a group that designs quality assurance systems for urinalysis products, and is also leading the team that will bring the Diagnostics R&D effort in Elkhart into ISO 9001 compliance.

Outside of Miles he is learning to become a storyteller, with special emphasis on stories from the distant past, as indicated by the opening of this article.