The within-run CV for duplicate counts was 10% or less. Interassay runs of the same controls or patients' specimens resulted in the same titers as previous runs. Therefore, interassay reproducibility was excellent.

The dynamic range without sample dilution was very limited. A titer of 1:32 could not be distinguished from a titer of 1:64 if we used the volumes and incubation conditions specified for the qualitative procedure. Titers of <1:8, 1:8, 1:16, and 1:32 correlated exactly with the HAI method. Titers >1:32 yielded a relatively flat response for radioactivity (cpm) vs titer. Of various dilutions we evaluated, a 10-fold dilution yielded the best response of cpm vs titer. The response between a titer of 1:256 and 1:512 was somewhat flat, but still usable.

The protein effect on dilution of calibrators, controls, and patients' specimens was insignificant with this titer system. Calibrators diluted with a negative control yielded the same titers as calibrators diluted with the kit assay buffer.

Rinsing the assay tubes with 0.5 mL of buffer three times after the first incubations of calibrators, controls, and specimens was changed to three rinses with isotonic buffered saline (pH 7.4) and an additional three rinses after the incubation with the tracer. This appeared to minimize background counts.

The first-step incubation with assay buffer and calibrators, controls, and specimens was maintained at 1 h at room temperature. No significant difference was noted when the incubation was done at 36 °C (air incubation). The second incubation with tracer showed significant differences with variation in reaction time. A 90-min incubation in a 37 °C water bath, which is part of the kit procedure, yielded a relatively flat response for titers exceeding 1:128. Overnight incubation (12 h) at 36 °C (air incubation) yielded excellent differentiation for titers between 1:128 and 1:256 with acceptable response between 1:256 and 1:512 and excellent response in the lower titers to 1:4. This incubation yielded count rates approximately twice those for the 90-min incubation, with no obvious change in nonspecific binding. This longer incubation interval appeared to result in a more complete reaction, and was necessary for acceptable differentiation and quantitation of titers at the high end of the scale.

For correlation studies we measured 32 specimens by the HAI method (Flow Laboratories, Inc., McLean, VA 22102) and 30 specimens by the indirect immunofluorescence method (IIF; International Diagnostic Technology, Santa Clara, CA 95051). Correlation with the HAI method yielded identical results for 24 of the 32 specimens and results were within one titer for the remaining eight specimens. Correlation with the IIF method was based upon interpretation of the IIF titer on a scale of <1:8, 1:8, 1:16, etc. Twenty of the 30 specimens gave identical results, and those for the other 10 specimens agreed within one titer. The titer range of specimens tested varied from <1:8 to >1:256. In summary, 62 specimens we analyzed yielded identical titers for 44 specimens (71%) by the GammaCoat RIA method and the remaining 18 specimens (29%) agreed within one titer.

Use of the GammaCoat RIA method required us to verify the Rubella Reference Serum (cat. no. CA334, lot no. 7-278), stated by Clinical Assays to be ≥1:160 HAI titer. We analyzed this calibrator in duplicate by the IIF method, the result being 1:160 for the HAI titer. The titer of the Rubella Reference Serum should be verified by the user. The calibrators are prepared as follows: four parts of 1:160 calibrator plus one part of assay buffer yields a 1:128 calibrator. This 1:128 calibrator is assigned a value of 1:1280 HAI titer units relative to a 10-fold dilution of patient and control specimens. The 1:1280 calibrator is then diluted with 1.5 parts of assay buffer to yield a titer of 1:512. Serial dilutions of the 1:512 with assay buffer yield HAI titers of 1:256, 1:128, 1:64, 1:32, 1:16, and 1:8.

All patients' specimens and controls are then diluted 10-fold with assay buffer. Each Rubella Antigen tube is labeled, in duplicate, and 200 μL of assay buffer is placed in each tube, followed by 10 μL of calibrators, controls, and patients' specimens. The contents of each tube are gently vortex-mixed and incubated at room temperature for 1 h. Then the contents are decanted, the inside of each tube is rinsed three times with 2 mL of buffered isotonic saline (pH 7.4, diluent used for blood-cell counters), 200 μL of tracer is added to each tube, and the tubes are incubated for 12 h (overnight) at 36 °C. After the 12-h incubation, all tubes are decanted and rinsed three times, as above, then the radioactivity is counted with a gamma counter for 1 min. With each run, a single nonspecific binding tube is included; the count for it approximates 300 (± 150) cpm. The count rate for a 1:8 titer is usually 2000 to 3500 cpm, with the count rate for the 1:512 titer exceeding by more than 10-fold the cpm of the 1:8 titer (e.g., 1:8 = 2300 cpm, 1:512 = 45 000 cpm).

The calibration titer points are established by averaging each duplicate calibrator. To establish HAI titer results, we average each patient's sample and control and compare this figure with the calibration points. We find this RIA method to be reliable, easy to perform, totally objective (as compared with HAI methods), and less expensive than most other methods.

We thank Mr. Albert Chapper of Medford Medical Laboratory, Inc., Medford, MA, and Drs. Louis Amoruso and Stanley Elfaiba of Clinical Science Laboratory, Inc., Sharon, MA, for supplying specimens and calibration data.

Reference
1. GammaCoat Rubella Antibody Radioimmunoassay Kit (cat. no. CA-538) Instruction Booklet, Cambridge, MA.

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Source of Error in β-Choriongonadotropin Determinations

To the Editor:

Pecchi et al. (Clin. Chem. 25: 335-336, 1979) reported an increased incidence of false-positive results for the hepatitis B surface antigen kit ("Austria-II"; Abbott Laboratories, North Chicago, IL 60064) because of carryover from the use of positive-displacement pipettes. They recommended the use of an air-displacement pipette to eliminate the problem.

I have encountered the same problem of false-positive results caused by carryover from highly positive specimens for the B-HCG kit (Clinical Assays, Cambridge, MA 02139) when using a positive-displacement pipette, and recommend the use of air-displacement pipettes for measurements of β-choriongonadotropin, especially considering the high concentrations of this hormone in the first trimester of pregnancy.

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A Lower Reference Interval for Hemoglobin A1c

To the Editor:

We have established in our hospital a reference interval for the Helena "Quik Column Total Fraction" method (1) for glycoylated hemoglobin (HbA1c) that is
considerably lower than that suggested by Helena Laboratories.

In this study, we closely followed the Helena procedure. Reagents, HbA\(_1\) controls, columns, collection tubes, pipets, and "Heme-Spec" spectrophotometer were all purchased from Helena Laboratories. Normal and abnormal screened controls were included in each run. Temperature was corrected to 22 °C when needed. Pipets, spectrophotometer (operated in Mode B), thermometer, and the procedure itself were repeatedly calibrated and checked by us and by technical representatives from Helena Laboratories. This was our third attempt to establish a new reference range for HbA\(_1\). The previous two involved smaller populations in which we only screened for glucose in blood and urine of fasting normal persons. Comparable lower results were observed in all three studies.

On correlating our results for HbA\(_1\) with clinical histories, we suspected that Helena's suggested reference range was too high. Results for many diagnosed and uncontrolled diabetics were normal according to Helena's range of 5.9 to 8.9%. At the same time, we were aware of three other hospital laboratories (two used Helena columns) who could not obtain a reference range as high as those suggested by the manufacturers of short-column HbA\(_1\) methods (2). One laboratory had actually stopped offering HbA\(_1\) testing because of confusion as to the reference range.

Our reference-range study was based on a small but carefully screened population of 64 pre-admission patients and volunteers. All had normal values for fasting glucose (0.65–1.15 g/L), cholesterol (1.5–3.0 g/L), SMA-16 screen, complete blood-cell count (hemoglobinopathy can cause abnormal HbA\(_1\) results), and urinalysis (except as trace of occult blood in urine from females). The pre-admission patients were to be admitted for the following specialties: obstetrics–gynecology, otolaryngology, orthopedics, urology, ophthalmology, plastic surgery, or general surgery. In this population, 17 were men and 47 were women. Their average age was 38, ranging from 16 to 84 years. All the selected hemoglobin were one day old and were stored refrigerated until use, except for complete blood-cell counts. The distribution plot of population vs percent HbA\(_1\) is essentially gaussian. At the 95% confidence level, we have a mean of 5.54 (2 SD = 1.52%), and a reference range of 4.0–7.1%. By using a tolerance interval to include 95% of the population at the 90% confidence level, the reference range would be 5.54 ± 1.70, or 3.8–7.2%. Either method of calculation yields a reference range that is considerably lower than that suggested by Helena Laboratories. In fact, even our mean is below the Helena's lower limit of 5.9%.

At the 95% confidence level, our new reference range of 4.0–7.1% is considerably lower than that suggested by most manufacturers of commercial kits for HbA\(_1\) by short-column methods. The discrepancy between the ranges may be due to different criteria used for population selection. Helena Laboratories used 70 adults, 18 years of age, who were presumed to be healthy "normal" individuals. We screened our fasting "normal" subjects to exclude anyone whose carbohydrate metabolism, complete blood-cell count, or results for general clinical chemical tests on blood and urine were abnormal.

We find that this lower reference range correlates very well clinically within our patient population.

References

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A spokesman for Helena Laboratories responds:

To the Editor:

We commend Lau et al. for their work and agree with their findings that normal ranges may vary from hospital to hospital as a result of many factors. For example, their sample population had a median age of 38 years, with a range from 16 to 84 years, while the median age in our study was 22 years, with a range of 18 to 30. Possibly the normal range was biased by this age discrepancy. In their study, samples were stored for one day at 2–6 °C. In our normal range, study samples were run within hours of collection. They also went to great lengths to determine the physical well-being of each patient. In their protocol, many other tests were run to determine that the patient was "normal." In our study we relied heavily on the patient's history and did not run any supplemental tests.

We do monitor our normal range and have, on a random basis, verified its accuracy. As yet, we have not noted a shift in our normal range that would warrant a major change in our published results.

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Phosphate Ion and Combined Phosphorus in Urine

To the Editor:

Ion chromatography is a relatively new analytical technique (1). Anderson (2) described its application to the determination of alkali earths, ammonium, phosphate, etc. in biological fluids. Phosphate ion in human urine can be so determined. We describe the determination of phosphate ion and combined phosphorus in urine and estimation of their excretion rate.

In the analysis for the phosphate ion, the Model 14 Ion Chromatograph (Dionex Corp., Sunnyvale, CA 94086) was used was equipped with a standard 3 × 150 mm precolumn, 3 × 500 mm anion separator column and 6 × 250 mm anion suppressor column. The eluent was 3 mmol/L NaHCO\(_3\)-2.4 mmol/L Na\(_2\)CO\(_3\). The flow rate was 115 mL/h. A 0.1-mL sample loop was used for all experiments. Concentration was determined from peak height. The urine sample was diluted about 300-fold with de-ionized distilled water. Before the sample was injected, a 10-mL plastic syringe containing a sterile filter (Millipore filter HA type, pore size 0.45 μm) was used to filter out suspended solids.

In the analysis for total phosphorus, 10 mL of urine was digested with 3 mL of sulfuric acid and 5 mL of nitric acid on an electric heater. Sometimes more nitric acid was needed for complete digestion. The digested sample was diluted to 25 mL with de-ionized distilled water and phosphorus was determined colorimetrically (3).

Five- to 600-fold dilution of sample had no effect on the dissociation or concentration of combined phosphorus in urine. The total phosphorus concentration in urine ranged from 0.78 to 3.6 g/L as PO\(_4^{3-}\). The range of phosphate ion concentration was 0.55 to 3.3 g/L for 86 residents of Nara. The average excretion was 1.4 g/L for phosphate ion and 1.6 g/L (as PO\(_4^{3-}\)) for total phosphorus. The difference between values for total phosphorus and phosphate ion can be defined as "combined" phosphorus in urine.

Figure 1 shows the cumulative distribution of phosphate ion (O) and total