The within-run CV for duplicate counts was 10% or less. Interassay runs of the same controls or patients' specimens yielded 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. Titters 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 titers 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, diluted 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, and 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 calibrator 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 Elbftein 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.

Joseph D. Musto
John M. Pizzolante
Vincent P. Chesarone
Newton-Wellesley Med. Lab., Inc.
Charles River Med. Center
25 Walnut St.
Wellesley Hills, MA 02181

Source of Error in β-Chorionic Gonadotropin Determinations

To the Editor:
Pecci 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-chorionic gonadotropin, especially considering the high concentrations of this hormone in the first trimester of pregnancy.

T. Higgins
T. A. Kaspar and Assoc.
10924—107 Ave.
Edmonton, Alberta T5H OX5
Canada

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