4 strips (Ames Division, Miles Labs., Elkhart, IN) is completely immersed in the diluted fecal extract and removed immediately. After a 2-min reaction time, the result is read by comparing the color on the strip with the color chart on the bottle (6). The result of the test, called the fecal LE titer, is the reciprocal dilution of the fecal extract that gives a score of one plus (+), i.e., ≤8, 8, 12, or a multiple of 8 or 12. The LE titer is 48 when the score in the 32-fold-diluted extract is more than + but the score in the 64-fold-diluted extract is less than +.

For an initial screening of fecal extracts from patients with suspected IBD, strips are dipped in extracts diluted 16-, 32-, 64-, and 128-fold. Additional strips are needed for samples with higher fecal LE titers. I determined the titers in 96 fecal portions obtained from 36 healthy laboratory workers or their relatives: 18 females, ages 5–67 years (mean 34), and 18 males, ages 7–89 years (mean 31). Because of the low titers in healthy donors, the initial dilution for these extracts was fourfold. The fecal LE titers determined for this group were ≤4 (n = 78), 8 (n = 9), 16 (n = 8), and 32 (n = 1).

Strongly increased titers are found more frequently in patients with large bowel disease than in those with small bowel disease. Fecal LE titers of 256 or higher were measured in 29 of 89 patients with large bowel inflammation and in 8 of 55 patients with small bowel inflammation (personal communication, I. Biemond, Department of Gastroenterology, University Hospital Leiden, The Netherlands). Further work is in progress to assess the clinical significance of fecal LE in patients with different diseases of the intestine and to study the correlation between fecal LE and other markers of disease activity.

The stability of fecal LE was studied in extracts with LE titers of 32 or higher. LE titers were determined directly and after incubation at 37 °C for 24, 48, 72, and 120 h. On the average, LE titers decreased by a factor of 1.4 (range 1–4, n = 24) after 24 h, 1.9 (range 1–4, n = 18) after 48 h, 2.0 (range 1–4, n = 14) after 72 h, and 3.8 (range 1–8, n = 18) after 120 h of incubation at 37 °C. Thus, the half-life of LE in fecal extracts is ~2–3 days. The half-life of lysozyme in similar extracts varied between 0.5 and 3.2 h (7).

The correlation between fecal LE and lysozyme was studied in 260 extracts of feces obtained from a random population of 140 patients with Crohn disease or colitis ulcerosa, either during active disease or during remission. Lysozyme concentrations were determined by means of a competitive enzyme immunoassay, with human lysozyme as a standard (8). These fecal extracts contained lysozyme concentrations between 0.04 and 90 mg/L and LE titers between 4 and 4096. There was a highly significant correlation between the two (Pearson's r = 0.753, P < 0.001). Discrepancies between fecal LE and fecal lysozyme may be related to the stability of the enzymes within the intestinal tract and to the origin of the enzymes from different cells involved in the inflammatory process. Fecal lysozyme appears to be derived mainly from granulocytes and Paneth cells in colitis ulcerosa and from macrophages in Crohn disease (9). As to the LEs, cytochemical studies show that different groups of isozymes occur in different cells: One group occurs predominantly in monocytes and macrophages, another group in granulocytes and their precursor cells (10). It is unknown, however, which esterase can hydrolyze the substrate in the LE reagent strips.

Because of the instability of lysozyme in the small bowel (7), it is possible to find a high fecal LE titer but a normal lysozyme concentration in patients with small bowel Crohn disease. For example, I measured a fecal LE titer of 2048 but a normal fecal lysozyme concentration in a patient with Crohn disease of the terminal ileum. During medical treatment, this patient's LE titer gradually decreased to 128.

In conclusion, the simple and rapid determination of fecal LE by means of dip-and-read reagent strips warrants further investigation as a method for monitoring the course of disease activity in patients with IBD.

References

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Calibration of the CYCLO-Trac SP Cyclosporine Radioimmunoassay

To the Editor:

Concern has been expressed about the accuracy of the calibrators supplied with the CYCLO-Trac SP radioimmunoassay kit (Inctar, Stillwater, MN) for measuring cyclosporine (1). These problems appeared to have been rectified by the manufacturer, and last year we were able to report results from the UK Cyclosporin Quality Assessment Scheme that showed good agreement between this method and other assays for cyclosporine, for measurements made in samples of known cyclosporine concentration (2). However, since compiling the latest complete survey of results from the UK scheme (3), we have noted a change in the performance of the CYCLO-Trac SP assay relative to the other assay techniques in common use, which, again, calls into question the accuracy of the calibrators supplied with this Inctar kit.

During the period covered by the latest report (3), July 1990 to July 1991, the accuracy of the methods for measuring samples with added cyclosporine (lot no. 88B252; Sandoz Pharma, Basel, Switzerland) to a known concentration was within ±4%
of the expected values for all the immunoassay techniques; HPLC assays gave results -6.5% below the expected values. However, last year, nine blood samples supplemented with cyclosporin to concentrations in the range 50-1100 µg/L were circulated as part of the UK Quality Assessment Scheme. The results for the analysis of these samples are summarized in Figure 1. The median deviation from the target values was -2%, +9%, and 0% for the EMIT assay (Syva, San Jose, CA), the HPLC assays, and the TDX monoclonal assay (Abbott Laboratories, Chicago, IL), respectively, but was +13% for the CYCLO-Trac SP assay. The percentage bias was positive over the concentration range, and the degree of bias was not related to the concentration measured.

The mean result for a blood sample circulated by the UK scheme in March 1993, with a nominal cyclosporine concentration of 500 µg/L, was significantly higher by the CYCLO-Trac SP than the three other methods (P <0.001, one-way ANOVA). The mean results (and number of centers reporting) were: 528 (7), 549 (79), 504 (20), and 617 (69) µg/L for HPLC, TDX, EMIT, and CYCLO-Trac SP assays, respectively. A frequency histogram of the CYCLO-Trac SP results for this sample made apparent a biphasic distribution of results, with modes of -525 and -485 µg/L. This distribution may be the result of the use of in-house calibrators by some centers, rather than those supplied with the kit, so that some values were closer to the nominal weighed-in concentration (500 µg/L). For instance, the result for this sample reported by our laboratory, using in-house calibrators prepared independently of the UK Cyclo-

sporin Quality Assessment Scheme, was 531 µg/L.

These findings have prompted us to reexamine the issue of calibration of the CYCLO-Trac SP assay, and we intend to revert to the practice of asking participants who are using this assay to indicate the source of their calibrators (2). By recording this information, we should be able to determine whether the change in the relative performance of this assay can be attributed solely to between-method differences in calibration.

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References


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An investigator for the manufacturer comments:

To the Editor:

Little is known about the factors that influence thiosulfate metabolism and excretion in humans. Concentrations in urine are greatly increased in sulfituria caused by various conditions (1), and colonic bacteria may have a role in the metabolism of urinary thiosulfate (2, 3). Thiosulfate is an intermediate metabolite in the oxidation of reduced sulfur products of bacterial metabolism (e.g., hydrogen sulfide). Endogenous pathways for the formation of thiosulfate have also been suggested (4).

We have developed a sensitive, simple colorimetric method based on a previously described ferric nitrate reaction (5, 6); the method is suitable for investigating some of the factors that influence thiosulfate excretion in humans. To remove endogenous interfering factors from urine, we use a Sep-Pak C18 cartridge (Waters, Milford, MA), washed consecutively with 2 mL of methanol and 4 mL of 10 mmol/L NaOH before use. Urine (10 mL) was adjusted to about pH 9.0 with 50 µL of 10 mol/L NaOH and centrifuged at 3000 x g for 10 min to remove particulate matter. Aliquots (2.2 mL) of supernate were passed through individual Sep-Pak C18 cartridges, and washed through with 1.0