Glycohemoglobin determination is valuable in managing diabetic patients as it allows an indirect measure of mean blood glucose levels in the preceding 2-3 month period. Glycohemoglobins are formed in a nonenzymatic fashion when glucose molecules react with amine residues on hemoglobin to form a ketoamine group. There are labile intermediate forms (aldimines) but the final step of ketoamine formation is irreversible, yielding a stable product. Methods for evaluating glycohemoglobin concentration include cation exchange chromatography, high-pressure liquid (boronate affinity) chromatography, and boronate affinity fluorescence polarization immunoassay. The "gold standard" for many years has been ion exchange chromatography. Early publications using this methodology related the glycohemoglobin A1c subfraction (Hb A1c) level to the degree of glycemic control in diabetics. This method was chosen as the reference method for the Diabetes Control and Complication Trial. The American Diabetes Association recommends the therapeutic goal of Hb A1c levels less than 7%.

There are limitations of ion exchange chromatography for glycohemoglobin determination. It relies on charge differences which occur following glycation of hemoglobin to separate hemoglobin fractions of hemoglobin A1. Hemoglobin charge can be affected by processes other than glycation (including variant hemoglobins such as Hb F and Hb S, labile glycohemoglobin intermediates, and temperature variation), leading to interference with the assay and misleading results. Boronate affinity based methods are specific for the ketoamine group formation and measure all glycohemoglobins (giving a more accurate picture of blood glucose levels over time). Accordingly they are replacing ion exchange chromatography in many laboratories (including Mayo Medical Laboratories). This may cause some confusion in interpreting the results of glycohemoglobin measured by a boronate affinity method.

The Rex Healthcare Laboratory uses a boronate affinity fluorescence polarization immunoassay (Abbott IMx™). Recently we have begun reporting a standardized % Hb A1c value in addition to the glycohemoglobin value. This is possible because of a study of 240 clinical specimens which demonstrated a linear relationship between the Abbott IMx™ method and the reference chromatography method.  

\[
\text{standardized } \% \text{ Hb A1c} = \frac{\text{glycohemoglobin} + 1.76}{1.49}
\]

The reference range for glycohemoglobin remains 4.8 -7.8%, while that for standardized % Hb A1c is 4.4-6.4%. It is our hope that this will assist physicians in relating their patients’ laboratory results with the "7% solution" recommended by the American Diabetes Association and others in the medical literature.

We would like to thank Dr. Selina James for inspiring this brief report!

John D. Benson, MD  
Deborah Brown, MT

Gastrointestinal Infections

Gastrointestinal infections encompass a wide variety of symptoms and recognized agents. A variety of tests are available to help to determine the causative agent. Diarrhea can be caused by one or more of several mechanisms.

- **Enterotoxins** altering the delicate balance of water and electrolytes in the small bowel, resulting in massive fluid secretion, “secretory diarrhea.” This is a noninflammatory process.
- **Cytotoxins** producing cell necrosis and/or a marked inflammatory response.
- **Infections** resulting in the penetration of the intestinal mucosa with subsequent spread to lymphatic or reticuloendothelial system outside of the gut.

An initial screen for fecal leukocytes is often suggested for the diagnosis of infectious diarrhea. This is based on the premise that the presence of fecal leukocytes is thought to be an indicator of inflammatory diarrhea caused by a variety of more serious pathogens.

The microscopic fecal leukocyte test has been available for years but has lately fallen to disfavor. The sensitivity of this test is reported to be about 73% for the diagnosis of Shigella but is less sensitive for other bacterial pathogens. In addition, fresh stools must be evaluated by experienced microscopists. This test is still available at Rex but the low sensitivity and lack of specificity make it a rather poor diagnostic tool.

A newer test, the lactoferrin test, has been shown to be more sensitive (93% for the detection of Salmonella, Campylobacter and Shigella). This is a commercially available latex agglutination test that measures lactoferrin found in leukocytes. The authors state, “The use of fecal lactoferrin to screen for inflammatory diarrhea selects specimens for which stool culture is fivefold more likely to yield an invasive bacterial pathogen and thus may greatly enhance a cost-effective approach to evaluating diarrheal illness.” Figure 2 shows a proposed algorithm for the use of fecal leukocyte screening for inflammatory diarrhea.

Stool cultures are still the definitive laboratory procedure to detect and identify bacterial pathogens. Standard cultures at Rex will detect Salmonella, Shigella and Campylobacter. Several other procedures can be performed to detect antigens or toxins associated with gastrointestinal disease. The following is a list of the most common causative agents and our recommendations for testing. We have separated these into groups based on the presence or absence of fecal leukocytes. Negative tests do not rule out the presence of fecal leukocytes because of the limited sensitivity of the currently available tests.

**Fecal leukocyte positive**

- **Salmonella/Shigella/Campylobacter** from ingestion of contaminated food or water. Causes dysentery. Order routine stool cultures.
- **E. coli O157** from ingestion of contaminated meat, especially hamburger. Causes bloody diarrhea. *When ordering must specify E. coli O157.*

**Fecal leukocyte positive or negative**

- **Clostridium difficile** follows disruption of intestinal flora as a result of antibiotic
treatment. Order C. difficile toxin. C. difficile cultures lack specificity and are no longer recommended.

- **Vibrio** from ingestion of contaminated water or shellfish. Causes watery diarrhea. *When ordering must specify Vibrio.*

**Fecal leukocyte negative**

- **Yersinia** from ingestion of contaminated milk, pork or water. Causes watery diarrhea. *When ordering must specify Yersinia.*
- **Giardia** from ingesting contaminated food, water. Causes watery diarrhea. Persistence (>10 days) with weight loss should prompt consideration of giardiasis. The most sensitive test is the Giardia antigen test done directly on stool.
- **Rotavirus** is most often seen in newborns and infants. Causes watery diarrhea. Order a Rotavirus antigen test.

The patient history, symptoms and duration of illness should be used to select appropriate testing and therapy (see footnotes 2 - 12 on the attached chart). The incubation period is typically 24 to 48 hours for most enteric bacterial pathogens but significantly longer for others such as Campylobacter (3 to 11 days) or E. coli O157 (3 to 5 days).

*Karl T. Kleeman, PhD*


Leukocyte reduction of blood products is equivalent to CMV seronegative units for prevention of CMV transmission by transfusion.

CMV (Cytomegalovirus) exposure is common in the population and usually causes mild or asymptomatic infection in immune competent people. About 50% of the adult population in North America is seropositive for antibodies to CMV, and in some areas up to 80% or more of the population is seropositive. However, CMV infection can cause serious disease in immunocompromised patients and is a recognized clinical concern for selected patients. In blood, CMV is found within leukocytes. It may cause either primary infection, reinfection, or reactivation of latent infection. Leukocytes are the primary site of latent infection and are probably the source of primary or reinfection in transfusion recipients. Leukocyte contaminants have been linked to CMV reactivation in the recipient.

Until recently, screening donated blood for antibody to CMV has been the only method of providing a transfusion option for decreasing the risk of CMV transmission. However, donor screening is difficult and logistically cumbersome because of the high prevalence of seropositivity. Furthermore, a recent study published in Transfusion (March 1998) found that cytomegalovirus DNA can be detected in leukocytes of most seronegative healthy blood donors. This important observation raises serious concerns about relying on seronegative donors for “CMV negative” units and probably explains reports of CMV reinfection/reactivation in recipients of CMV seronegative blood.

If CMV resides in leukocytes, can removal of the leukocytes impact CMV transmission? Yes. Eight uncontrolled clinical trials and a controlled marrow transplant study have provided strong evidence that CMV transmission could be prevented by leukoreduction. In addition, a large multi-center prospective clinical trial has demonstrated that leukocyte reduced blood components (using the leukocyte filter technology employed at Rex) are clinically equivalent to screened CMV seronegative blood components. This very important study supports the mounting evidence that leukocyte reduced blood products, prepared by either bedside filtration or prestorage leukocyte depletion, can be used as equivalent to specially screened seronegative units.

Patients at risk for morbidity from CMV transmission include immunocompromised patients such as premature neonates, transplant patients, CMV seronegative pregnant women, HIV positive patients, cardiac surgery patients, and some patients undergoing chemotherapy. Most of these patients will also benefit from leukocyte reduced blood products for other reasons. It may be that ordering “CMV negative” units will rapidly be replaced by ordering leukocyte reduced blood products for this and many other reasons. Indeed, as the indications for leukocyte reduction continue to expand, the time when all blood should be leukocyte reduced may have arrived.

Timothy R. Carter, MD

Larsson et al. Cytomegalovirus DNA can be detected in peripheral blood mononuclear cells from all seropositive and most seronegative healthy blood donors over time. Transfusion 1998;38:271-278.


For further information, call the Laboratory (784-3040). Telephone extensions are: Pathologists' Direct Line (3201), Dr. Kleeman (3063), Sharon Logue (Lab Director 3055), Robin Ivosic (Core Lab Manager 3053), Linda Lompa (Blood Services Manager 785-4770), Kimberly Skelding (Customer Services Manager 3318), Rex Outreach (784-3040), Karen Sanderson (Lab Compliance Specialist 3396).