Clinical Utility of the IRF: Assessment of Erythroid Regeneration Following Parvo B19 Infection

JANIS WYRICK-GLATZEL, JANICE CONWAY-KLAASSEN

Parvo B19 (Fifth disease) is an erythrotropic virus which attaches through the 'P' globoside receptor on the surface of human red blood cells and precursors. This typically benign viral infection can cause a transient aplastic anemia in patients with underlying red cell disorders. In this case, a two-year-old child presents with severe aplastic anemia without evidence of underlying disease. Erythroid regeneration is monitored through the use of the immature reticulocyte fraction (IRF) and is demonstrated by the presence of high and medium fluorescence reticulocytes in the peripheral blood three to five days prior to the peak in absolute reticulocytes.

ABBREVIATIONS: IRF = immature reticulocyte fraction.

INDEX TERMS: erythroid hypoplasia; erythroid marrow regeneration; flow cytometry; globoside-p antigen; hemolytic anemia; parvovirus B19 infection; reticulocytes.

Clin Lab Sci 2002;15(4):208

Janis Wyrick-Glatzel MS *is Associate Professor, Clinical Laboratory Science Program at the University of Nevada Las Vegas, Las Vegas NV.*

Janice Conway-Klaassen MS CLS(NCA) *is Assistant Professor and Program Director, Clinical Laboratory Science Program at the University of Nevada Las Vegas, Las Vegas NV.*

Address for correspondence: *Janis Wyrick-Glatzel MS, Clinical Laboratory Science Program, Mail Stop 3021, University of Nevada Las Vegas, 4505 S Maryland Parkway, Las Vegas Nevada, 89154-3021. (702) 895-0977/3788, (702) 895-3872 (fax). jglatzel@ccmail.nevada.edu*

Parvovirus B19 (B19) is a species specific, small, nonenveloped, single-stranded DNA virus that belongs to the family *Paroviridae*, genus *Erythrovirus*. Discovered by Cossart and colleagues in 1975, it is the only known human pathologic parvovirus associated with a broad spectrum of disorders.[1] Human B19 is considered a ubiquitous virus with distribution worldwide. Cultured from the respiratory tract, Parvovirus B19 is presumed to be transmitted as an aerosol. Prevalence of infection is common, reported at a frequency ranging from 2% to 15% in children one to five years of age, approximately 15% in children ages five to 19 years, with approximately 50% of adults testing seropositive.[2] Diverse clinical manifestations arise due to B19 infection. Infection follows a cyclic pattern with increased rates occurring every four to five years.[3] Infection can present as asymptomatic, cause *erythema infectiosum*, or induce a polyarthropathy syndrome, a hemolytic anemia, or hydrops fetalis during pregnancy. Severe anemia due to bone marrow aplasia is often a major complication as a result of viral replication in erythroid precursors. The most common clinical manifestation, *Erythema infectiosum* or Fifth disease is a benign, self-limiting febrile illness associated with marked erythema of the cheeks, known as 'slapped cheek', and a lacy rash on the trunk and extremities. A migratory polyarthropathy syndrome, immunologically mediated, may occur in infected children and adults, particularly women, with symptoms generally persisting for one to three weeks. By the time the rash or polyarthropathy presents in immunocompetent patients, viremia has cleared and the presence of serum antibodies confirms the diagnosis. Studies have shown that patients with an underlying chronic hematologic disease, such as sickle cell anemia, or those with immunodeficiency states, such as HIV, are at risk for severe anemia with transient aplastic crisis of the bone marrow.[4,5]

Parvovirus B19 has a predilection for replication in the bone marrow erythroid progenitor cells including the erythroid colony-forming units (CFU-E) and the burst-forming units (BFU-E).[6] Infection suppresses erythropoietic activity of the bone marrow. The B19 virion consists of capsid proteins and DNA genome. The capsid proteins give stability to the B19 virus. Studies have shown that the P blood group antigen, a globoside, serves as the receptor for B19 capsid attachment.[7] Globoside is found on cells within the bone marrow such as early erythroid progenitor cells, megakaryocytes, and on cells within the placenta, fetal myocardium, kidney, and thyroid. Myocarditis contributes to the hydrops fetalis associated with B19 infection. Studies have shown that rare individuals who lack the P antigen and therefore lack the receptor for the virus are not susceptible to B19 infection.[8] Viral replication, by means of host cellular DNA polymerase, occurs within the nucleus of actively dividing cells, following the S phase of the host's cell cycle. The palindromic sequences of the genome enables the molecule to fold over on itself and form a hairpin structure important for replication. The 5 kb genome of the human B19 parvovirus

The Reports and Reviews Section seeks to publish information on important clinical laboratory-related topics such as technological, clinical, and experimental advances and innovations. Case studies and literature reviews are also included. In addition, brief reviews of books, computer programs, audiovisual materials or other materials of interest to readers are appropriate for this section. Manuscripts and literature reviews published as a Report are peer reviewed. Direct all inquiries to Isaac Montoya PhD, Affiliated Systems Corporation, 3104 Edloe, Suite 330, Houston TX 77027-6022. (713)439-0210, (713)439-1924 (fax). imontoya@affiliatedsystems.com

encodes for structural proteins VP1 and VP2 and the non-structural proteins NS1 and NS2.[9] The infection of susceptible cells and the expression of non-structural protein NS1 has been shown to impair cellular mechanisms and promote apoptosis of erythroid progenitors.[10,11] Cell death can occur without viral replication because the production of the nonstructural proteins are cytotoxic. Immunologic suppression of bone marrow erythropoiesis has also been reported to occur associated with B19 infection.[11] Individuals at risk for prolonged B19 infections are those who do not develop neutralizing antibodies to VP1 as measured in erythroid progenitor assays.[12]

A hemolytic anemia associated with transient bone marrow erythroid hypoplasia is a serious complication of Parvovirus B19 infection. Infected cells fail to proliferate and mature thus prohibiting the production of new red blood cells. Bone marrow examination reveals dyspoietic changes to pronormoblasts with few mature normoblasts present. Reticulocytopenia is the cardinal sign of an aplastic crisis.

Disturbances in the dynamic equilibrium of erythropoiesis can be monitored clinically by the absolute reticulocyte count and the maturity of the subpopulations of reticulocytes quantified by flow cytometry. Red blood cell survival studies in normal individuals show the lifespan of a reticulocyte to be one to two days in peripheral circulation. During periods of increased erythropoiesis, usually accompanied by increases in erythropoietin, the lifespan of a reticulocyte in circulation is increased to three or more days, owing to the release of 'stress or shift' reticulocytes from the bone marrow and accelerated erythroid differentiation. The quantity of RNA found in reticulocytes is a measurable biochemical change within the cell that can define the maturity of the various subpopulations in circulation. Bone marrow 'stress or shift' reticulocytes are primarily defined by the high amount of RNA found within the cell. Thus, the ability to not only count but to fractionate reticulocyte populations based on RNA content into levels of maturity has significant clinical application in assessing erythropoietic activity.

The Sysmex R-series is a fully automated, multiparameter flow cytometer that utilizes light scatter and the fluorescent dye Auramine-O (AuO) to bind in a stoichiometric fashion to the RNA within the reticulocyte (Sysmex Corporation, Gilmer Road 6699, Long Grove, IL 660047, a subsidiary of TOA Medical Electronics CO., LTD, 1-800-3-SYSMEX, www.sysmex.com). The use of this methodology permits reticulocytes to be counted and discriminated based on the content of RNA in the cell. Fluorescence is divided into three regions known as Low Fluorescent Reticulocytes (LFR), Medium Fluorescent Reticulocytes (MFR), and High Fluorescent Reticulocytes (HFR). Highly fluorescent reticulocytes contain the most cellular RNA and represent those reticulocytes recently released from the bone marrow into the peripheral circulation. The quantification of RNA in reticulocytes has generated a useful clinical parameter known as the immature reticulocyte fraction or IRF. This parameter has significant clinical application in assessing regenerative and nonregenerative hematologic conditions. The IRF, is defined as the sum of the HFR and MFR region and as such is the standard nomenclature recommended by NCCLS guidelines.[13,14] In the Sysmex R series analyzers, the sum of HFR and MRF demonstrates an asymmetric log-normal distribution, thus making an increased IRF value of clinical significance.[13] Studies have demonstrated that the IRF of bone marrow is consistently higher than that of peripheral blood with a mean of 0.41 versus 0.34 respectively, owing to the shift toward the more immature and RNA rich proportion of reticulocytes in the bone marrow.[15] Bivariant analysis of the IRF and the absolute reticulocyte count provides data to assess the adequacy of the erythropoietic response for the level of anemia as well as a classification scheme for anemia as demonstrated in various studies.[16] The presence of both a low absolute reticulocyte count and a low IRF suggests a reduced level of erythropoiesis associated with aplastic or hypoplastic phases. Increased erythropoietic activity following an aplastic crisis generally manifests with normal to increased IRF with below normal reticulocyte counts. Patients with a compensated hemolytic anemia present with increases in both the reticulocyte count and the IRF.[16]

CASE PRESENTATION

A 25-month-old male Caucasian child was brought to the emergency room by his mother. Physical examination revealed an afebrile, pale, lethargic child. Skin and mucous membranes were pale and gums blanched. Clinical history revealed a mild flu-like illness accompanied by a low-grade fever over the past week. During this time, the child had been in day-care for three days. There had been no major illnesses nor were there any underlying disorders noted. All immunizations were complete and up-to-date. The child appeared of average weight and size for his age. The patient was admitted to the hospital the same day. Complete blood and chemistry profiles were ordered.

Laboratory results

On admission, results were as follows: WBC 6.9 x 10^9/L; RBC 1.26 x 10^{12}/L; hemoglobin 3.9 gm/dL; hematocrit 11.8 %, and absolute reticulocytes 19.0 x 10^9/L (Table 1). Values between 10 and 110 x 10^9/L are considered as the normal absolute reticulocyte reference range according to NCCLS Standards.[17] Sysmex R Series reference ranges are 24-84 x 10^9/L. The differential showed 50 PMNs, 7 bands, 37 lymphs, 5 monocytes, and 1 metamyelocyte. Serum chemistries were essentially normal with the exception of total bilirubin and LD (Table 2). Blood bank results showed the patient to be group 'O' with a positive DAT, all cells positive on antibody screen (with IgG Coombs), and an inconclusive Rh typing. A unit of packed red blood cells aliquoted into two bags was ordered for transfusion. The patient was transfused following a biologic crossmatch in an attempt to maintain hemoglobin and hematocrit levels. Additional laboratory tests ordered included: urinalysis, CMV cultures, hepatitis profile, and serologic tests for CMV and Parvo B19.

Table 1. Hematology results during hospitalization and clinic follow-up

	DAYS OF ADMISSION										WEEKS AFTER DISCHARGE			
	1	2	3	4a*	4b*	5	6	8	10	12	15†	1	2	3	4
RBC‡ (10^{12}/L)	1.26	0.98	1.08			2.44	2.34	1.86	2.18	1.70	2.19	3.22	3.89	3.85	4.06
HGB (gm/dL)	3.9	2.7	3.2	2.2	6.7	6.2	6.1	4.7	5.7	4.6	6.9	10.0	12.1	12.0	12.0
HCT (%)	11.8	8.0	9.3	6.1	19.5	17.6	17.0	13.8	17.4	15.2	23.4	32.0	36.9	35.0	34.4
RETIC‡ (%)	1.51	0.3	0.28			0.2	0.41	1.8	7.31	16.61	28.6	11.04	3.61	1.62	1.0

* (a) pre-transfusion results; (b) post-transfusion
† Day of discharge
‡ RBC and reticulocyte counts taken from Sysmex Series

Table 2. Chemistry results during hospitalization and clinic follow-up

	DAYS OF ADMISSION					WEEKS*
	1	3	5	10	12	1
LD (IU/L)	1335	1417	1476	1566	1517	712
T Bilirubin (mg/dL)	12.9	4.8	3.9	4.3	4.0	1.3
D Bilirubin (mg/dL)	0.1	0.9	0.0	0.1	0.1	0.0
T Protein (mg/dL)	6.5	8.7	9.1	8.9	7.7	7.1
Albumin (gm/dL)	3.9	4.0	3.6	4.2	3.7	4.1
Globulin (gm/dL)	2.6	4.7	5.5	4.7	4.0	3.0

* Weeks after discharge

Clinical case report

Subsequent to admission, the child was hospitalized for 15 days, released, and followed at the clinic during which time the following pertinent laboratory data was compiled (Tables 1, 2, and 3). During the second week of hospitalization, the child presented with a pronounced erythematous rash primarily involving the cheeks of the face, upper trunk, arms, and legs. In the two-day period following admission and in vivo crossmatch, the patient's red cell counts, hemoglobin, hematocrit, and absolute reticulocytes continued to fall (Tables 1 and 3). Serum chemistries showed significant elevations in lactate dehydrogenase (LD) and total bilirubin, with alterations in the total protein, albumin and globulin fractions (Table 2). Rises in LD and total bilirubin with a continual drop in the hemoglobin and hematocrit were consistent with a noncompensated hemolytic crisis. Urinalysis results were unremarkable except for the presence of two WBCs per high power field. Results on initial blood and urine samples for CMV were negative.

Noting all major abnormal laboratory findings, a presumptive diagnosis of immune mediated hemolysis accompanying Fifth's disease was made. An eluate was performed with all panel cells reactive with IgG Coombs. A second biologic crossmatch was performed and a second unit of packed red cells was issued and transfused on day four following admission. Following the second transfusion of packed red cells, post-transfusion rises in the hemoglobin and hematocrit were achieved. LD and total protein continued to rise however, reaching 1566 IU/L, and 8.9 mg/dL respectively on day ten following admission and red cell transfusion. Total bilirubin decreased significantly following admission and remained mildly elevated throughout hospitalization (Table 2).

An absolute peripheral blood reticulocytopenia classically defining bone marrow erythroid hypoplasia ensued for six days as documented by the absolute reticulocyte count (Table 3).

A blood specimen was analyzed for Parvovirus B19 antibody 23 days following admission and presentation to the clinician. Testing revealed IgM levels of 2.58, and IgG levels of 2.74; values >1.20 are interpreted as positive. Both IgM and IgG were positive, consistent with current or recent infection within the last two to three months.

A follow-up specimen drawn approximately two weeks after the first specimen tested below threshold values for Parvo B19 IgM (1.13) but positive for Parvo B19 IgG (2.26).

The decrease of IgM antibody and the persistence of IgG antibody correlates with recovery from recent infection and immunity. In general, viremia precedes the onset of Fifth

disease and the associated transient aplastic crisis. Although no actual viral serology was done during the acute phase of the disease, the globulin level on day five was significantly elevated (5.5 gm/dL), most likely indicative of a higher antibody titer. Blood bank results support the evidence of an immune response as seen by the positive DAT on admission and a positive IgG eluate. Following the peak in globulin on day five, recovery of bone marrow reticulocytes is evident by way of increasing absolute reticulocyte counts (Table 3). The assumption is that viral particles and infected cells are now being cleared from the system thus allowing bone marrow regeneration of reticulocytes and maintenance of the peripheral blood red cell count (Table 3; Figure 1). Review of the peripheral blood differential indicated an absolute lymphocytosis concurrent with the globulin peak.

Hematologic findings in this pediatric patient demonstrated an immune hemolytic anemia with an aplastic crisis of the bone marrow as evidenced by the use of the IRF and the absolute reticulocyte count (Table 3; Figure 1). The IRF directly assesses reticulocyte maturity; the absolute reticulocyte count accounts for the severity of the anemia; and the combined use evaluates the adequacy of the marrow erythropoietic response. In general, it has been noted that patients with a severe anemia or significant reticulocytosis as defined by increases in absolute reticulocyte count also had increased IRF. Hypoplastic or aplastic crisis are defined by decreases in both the absolute reticulocyte count and the IRF. Sequential measurements of the IRF and absolute reticulocyte counts in bone marrow transplant patients demonstrate a reproducible pattern highlighting early signs of erythroid regeneration within the bone marrow.

DISCUSSION

The diagnosis of human Parvovirus is generally determined by the serologic presence of IgM and/or IgG antibodies and a concomitant hemolytic anemia as demonstrated by decreasing hemoglobin and absolute reticulocyte counts. In general, transient aplastic crises are common often requiring supportive care and transfusion. Pregnant women, immunocompromised individuals, and those with chronic hemolytic anemias are at the highest risk for complicated parvovirus infection. Viral titers are usually elevated during clinical manifestations of the disorder. IgM antibody against the virus rises and generally peaks within 14 days after the onset of the disease followed by a rise in the IgG antibody. Immunity is considered lifelong following infection.

Bone marrow infection manifests by a transient erythroid hypoplasia which morphologically presents as dysplastic, megaloblastic pronormoblasts with intra-nuclear bodies. Parvovirus B19 has a strong tropism for, and cytotoxic properties against, erythroid progenitors preventing cellular replication and maturation. Studies demonstrate that a non-structural gene protein promotes apoptosis of the infected erythroid cell line. The P antigen, a globoside found on erythroid progenitor cells, serves as cell receptor for viral attachment.

The associated hemolytic anemia and transient aplastic crisis has classically been monitored by the peripheral reticulocyte count reported as either the percent reticulocytes or absolute reticulocytes (10^9/L). The IRF has been shown to increase three to five days prior to the increase seen in reticulocyte counts and therefore can assess marrow erythropoietic activity earlier than the reticulocyte count. The IRF is an early and sensitive indicator of erythropoiesis. Those patients with a severe degree of anemia are expected to demonstrate an increased IRF with decreased absolute reticulocyte counts, indicative of the expected physiologic response of increased erythropoiesis. In patients with a chronic hemolytic ane-

Table 3. Reticulocyte results during hospitalization and clinic follow-up*

	DAYS OF ADMISSION									WEEKS AFTER DISCHARGE			
	1	2	3	5	6	8	10	12	15†	1	2	3	4
ABS RETIC (10^9/L)	19.0	3.3	3.0	4.9	9.6	33.5	159.4	282.4	626.3	355.5	140.4	62.4	41.6
IRF	11.0	2.5	25.0	33.3	57.9	75.6	67.8	49.5	28.0	18.5	10.4	8.2	2.6
HFR	0.4	0.0	2.8	22.8	26.3	56.1	24.9	12.7	5.5	2.3	0.7	0.4	0.0
MFR	10.6	2.5	22.2	10.5	31.6	19.5	42.9	36.8	22.5	16.2	9.7	7.8	2.6
LFR	89.0	97.5	75.0	66.7	42.1	14.4	32.2	50.5	72.0	81.5	89.6	91.8	97.4

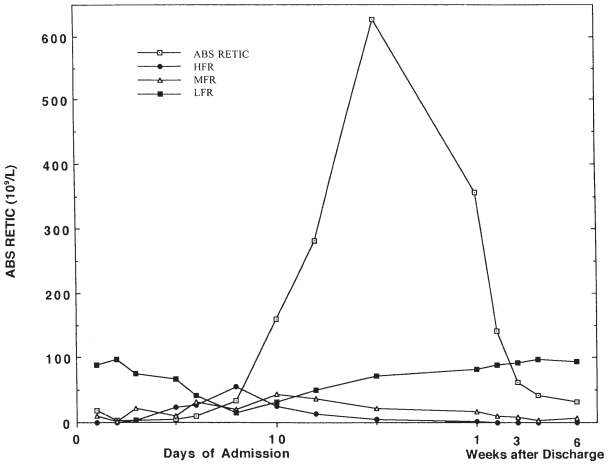
* Reticulocyte counts taken from Sysmex Series
† Day of discharge
IRF = immature reticulocyte fraction (HFR + MFR)
HRF = high fluorescence reticulocyte fraction
MRF = medium fluorescence reticulocyte fraction
LRF = low fluorescence reticulocyte fraction

mia, there is an increase in the IRF as well as an absolute reticulocytosis. Severe hypoplasia results in a decrease in both the IRF and the absolute reticulocyte count. Bivariant analysis of the absolute reticulocyte numbers and the IRF aid to further define the adequacy of the response.

Serial measurements of the IRF has practical significance in its ability to assess the status of engraftment during the post-transplant period. Early studies show that the absolute reticulocyte counts and the reticulocyte percent were insensitive in predicting engraftment. The first response following bone marrow ablation is an increase in the IRF which generally precedes the increase in the reticulocyte count. With the increased sensitivity and clinical utility of the IRF, a better means of monitoring the regenerative bone marrow response following an aplastic crisis or stem cell transplantation has evolved.

Clinical utility of the IRF not only has application in the evaluation and classification of anemia as a result of erythroid hypoplasia, but in determining therapeutic protocol and efficacy of erythropoietin treatment in chronic renal failure and in assessing hematopoietic regeneration following chemotherapy, radiation, or bone marrow or peripheral blood stem cell transplantation. The IRF also has use in monitoring the therapeutic response to iron, folic acid, vitamin B12, and is useful in redefining apheresis protocols when harvesting peripheral stem cells.

Figure 1. Comparison of absolute reticulocytes vs. fluorescent reticulocyte fractions



Fluorescent reticulocyte (%) counts taken from Sysmex Series Analyzer
HFR = high
MFR = medium
LFR = low

SUGGESTED READINGS/WEB SITES

http://www.cdc.gov/ncidod/diseases/parvovirus/b19.htm
http://www.hhs.state.ne.us/epi/epi5thd.htm
http://www.stanford.edu/group/virus/parvo/parvovirus.html
http://www.biotrin.ie/products/infectious_diseases/b19/booklet.html
http://www.beckmancoulter.com/coulter/techpubs/hematology/pe-retics.asp
http://medlineplus.adam.com/ency/article/003637.htm
http://www.cyto.purdue.edu/flowcyt/educate/aautoretsld020.htm
http://www.tulane.edu/~dmsander/WWW/335/Parvoviruses.html
http://www-micro.msb.le.ac.uk/335/Parvoviruses.html

REFERENCES

1. Cossart YE, Field AM, Cant B, and others. Parvovirus-like particles in human sera. Lancet 1975;1:72-3.
2. Anderson LJ: Role of parvovirus B19 in human disease. Pediatr Infec Dis J 1987;6:711.
3. Brown KE, Young NS, Barbosa LH. Parvo B19: implications for transfusion medicine. Summary of a workshop. Transfusion 2001;41:130-5.
4. Rao SP, Miller ST, Cohen BJ. Transient aplastic crisis in patients with sickle cell disease. B19 parvovirus studies during a 7 year period. Am J Dis Child 1992;1328-30.
5. Frickhofen N, Abkowitz JL, Safford M, and others. Persistent B19 parvovirus infection in patients infected with human immunodeficiency virus type 1 (HIV-1): a treatable cause of anemia in AIDS. Ann Intern Med 1990;113:926-33.
6. Takahashi T, Ozawa K, Takahashi K, and others. Susceptibility of human erythropoietic cells to B19 parvovirus in vitro increases with differentiation. Blood 1990;75:603-10.
7. Brown KE, Anderson SM, Young NS. Erythrocyte P antigen: cellular receptor for B19 parvovirus. Science 1993;262:114-17.
8. Weigel-Kelley KA, Yoder MC, Srivastava A. Recombinant human parvovirus B19 vectors: erythrocyte P antigen necessary but not sufficient for successful transduction of human hematopoietic cells. J Virol 2001;75:4110-6.
9. Ozawa K, Young N. Characterization of capsid and noncapsid proteins of B19 parvovirus propagated in human erythroid bone marrow cell cultures. J Virol 1987;61:2627-30.
10. Sol N, LeJunter J, Vassias I, and others. Possible interactions between NS-1 protein and tumor necrosis factor alpha pathways in erythroid cell apoptosis induced by human parvovirus B19. J Virol 1999; 3:8762-70.
11. Brown KE, Young NS, Liu JM. Molecular, cellular, and clinical aspects of parvovirus B19 infection. Crit Rev Oncol Hematol 1994;16:1.
12. Kurtzman GJ, Cohen BJ, Field AM, and others. Immune response to B19 parvovirus and an antibody defect in persistent viral infection. J Clin Invest 1989;84:1114-23.
13. Davis BH. Immature reticulocyte fraction (IRF): by any name, a useful clinical parameter of erythropoietic activity. Lab Hematol 1996;2:2-8.
14. National Committee for Clinical Laboratory Standards: Methods for reticulocyte counting: flow cytometry and supravital dyes. Approved guidelines. Document H44-A. Villanova PA: NCCLS 1997.
15. D'Onofrio G, Zini G, Tommasi M, and others. Quantitative bone marrow analysis using the Abbot CELL-DYN 4000 hematology analyzer. Lab Hematol 1997;3:146-53.
16. Davis BH, Ornvold K, Bigelow NC. Flow cytometric reticulocyte maturity index (RMI): a useful laboratory parameter of erythropoietic activity in anemia. Cytometry 1995;22:35.
17. Buttarello M, Bulian P, Farina G, and others. Flow cytometric counting: parallel evaluation of five fully automated analyzers: An NCCLS-ICSH Approach. Jan. 2001. Available at http://www.ajcp.com/buttarello0101ar.htm. Accessed 02/27/01.