Use of the Quantitative Buffy Coat System for Detection of Parasitemia in Patients with Babesiosis

ANTHONY R. MATTIA,* MARY ANN WALDRON, AND LUZ STELLA SIERRA

Parasitology Section, Clinical Microbiology Laboratory, and the Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

Quantitative Buffy Coat analysis and blood smears were performed on a total of 47 blood samples. The technique showed 100% correlation with the blood smears in 9 samples containing babesia and 10 samples containing malaria, with some differential features distinguishing the two infections. Quantitative Buffy Coat analysis provides a simple and rapid method for the detection of parasitemia in cases of babesiosis.

Human babesiosis is an increasingly recognized zoonotic malaria-like illness caused by an intraerythrocytic protozoan parasite that is transmitted by nymphal *Ixodes dammini* ticks (7, 13, 17, 25) or, rarely, by transfusion (18, 21). *Babesia microti* has been the predominant human pathogen in the United States and is endemic in a circumscribed geographic area of the northeast coastal United States (14, 23). Isolated infections from other regions (8, 25, 26) indicate a potential for the expanding range of this parasite.

Most cases of babesiosis are asymptomatic or mild and have a low-grade parasitemia (8, 17, 23). Clinically apparent disease is more frequent in elderly individuals, or in persons without a functioning spleen, who often have a high level of parasitemia and more severe symptomatology (8, 9, 12, 27). In AIDS patients, babesiosis may present as a recurrent disease that requires prolonged therapy (4). Fatalities due to *B. microti* are rare.

The symptoms of babesiosis are nonspecific, and clinical diagnosis is difficult. Definitive laboratory diagnosis generally requires direct observation of parasites in Giemsa-stained peripheral blood films. However, since parasites are typically scarce, the blood smear is relatively insensitive (19). Supplemental methods such as amplification by hamster inoculation (5), serology (6), and parasite detection by the polymerase chain reaction (19) currently play a limited role in individual case diagnosis.

Recently, various investigators have documented the utility of the commercially available Quantitative Buffy Coat system (QBC; Becton Dickinson, Tropical Disease Diagnostics Division, Sparks, Md.) for the diagnosis of malaria and other blood-borne parasites (1, 3, 10, 15, 22, 24). This technique utilizes acridine orange staining of parasites within specialized capillary tubes, differential centrifugation, and visualization with fluorescent epi-illumination and has been shown to be more rapid and often more sensitive than the thick blood smear for parasite detection. We undertook this study to determine whether QBC analysis would be similarly applicable as a rapid method for parasite detection in human babesiosis, which has not been previously reported.

A total of 47 peripheral blood samples from 32 patients were obtained with EDTA anticoagulant from the following sources: (i) 39 consecutive, prospectively evaluated samples received over a 3-month period at the Parasitology Laboratory of the Massachusetts General Hospital from patients

suspected of having malaria or babesiosis; (ii) two consultation samples; (iii) one selected *Plasmodium vivax*-containing sample; and (iv) five samples submitted to the Department of Tropical Public Health, Harvard School of Public Health, Boston, Mass., from previous parasitologically confirmed cases of *B. microti* infection from Nantucket Island. Blood was examined the same day it was drawn or stored at 4°C until further analysis.

Giemsa-stained thick and thin blood smears were examined by conventional light microscopy on all specimens processed in the Massachusetts General Hospital Parasitology Laboratory, and only thin smears were examined for consultation and Harvard School of Public Health samples. Cases of babesiosis were identified by the presence of characteristic parasite morphology on the blood smears (7, 11, 27), in conjunction with an appropriate clinical history. Malaria cases were similarly identified by morphologic characteristics (2), along with clinical correlation. Percent parasitemia was estimated in all positive cases by counting 20 randomly chosen thin smear fields containing a single layer of nonoverlapping cells. Cases with negative peripheral blood smears served as controls. Negative smears required evaluation of 500 oil immersion fields (on two slides per sample) over approximately 20 to 30 min.

Following the preparation of the Giemsa-stained thick and/or thin blood smears, a portion of the specimen was examined with the QBC method as described by the manufacturer. Acridine orange-coated capillary tubes containing 55 to 65 ml of blood were centrifuged at 12,000 rpm (14,367 × *g*) for 5 min in a centrifuge provided by the manufacturer. The resultant density gradient separates and concentrates the blood cells (Fig. 1), parasitized erythrocytes, and extracellular parasites relative to their stage of maturity and/or species (22). The tubes were examined in a specialized tube holder via UV epifluorescence provided by the UV ParaLens Microscope Adapter (Becton Dickinson). This device consists of a high-intensity halogen light source and a fiber optic cable attached to a specialized objective adapter that can be inserted into a spare lens port on any standard light microscope (16, 20). The entire cell column was examined for fluorescing parasites; determination of a negative sample required approximately 5 min.

Both blood smears and QBC analysis demonstrated *Babesia* organisms in 9 samples from seven patients and *Plasmodium* species in 10 samples from six patients (Table 1). No false positives or negatives were observed.

The seven patients with babesiosis had a relevant history

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* Corresponding author.

FIG. 1. Schematic diagram of the QBC capillary tube showing the layering characteristics of blood cell components after centrifugation (RBC, erythrocyte layer; PMN, polymorphonuclear leukocyte layer; L/M, lymphocyte-monocyte layer; PLT, platelet layer).

of potential exposure on the island of Nantucket (six patients) or Martha's Vineyard (one patient), and none were asplenic. Both the Giemsa-stained thick and thin smears were positive for two samples from two patients, and for the remaining seven positive samples, only thin smears were evaluated. The degree of parasitemia was low grade. QBC examination of the four fresh samples disclosed abundant small, brightly fluorescing extracellular parasites concentrated in a thin band immediately above the platelet layer, in addition to numerous intraerythrocytic parasites that were scattered throughout the erythrocyte layer. One sample with 5% parasitemia showed a slight concentration of parasitized cells in the upper erythrocyte layer. The five blood samples examined 8 to 17 days after being drawn contained several artifacts that altered the localization of parasites within the tube, such as aberrant layering due to small fibrin clots, hemolysis, and cell debris. However, brightly fluorescing organisms were easily found scattered throughout the erythrocyte layer in all five samples, with a moderate concentration of organisms in the residual platelet layer in one sample. The predominant morphologic appearance of *Babesia* organisms with the QBC system was a tiny bright chromatin dot surrounded by a small round or pyriform-shaped body of cytoplasm. The QBC allowed the detection of parasitemia within 30 to 60 s in all cases, compared with up to 30 min required to locate scarce parasites on several of the thin smears.

The malaria patients had a history of recent travel and/or residence in malarious areas. QBC analysis was performed within 24 h for all 10 samples, and the samples exhibited layering, concentration, and morphologic features as described by previous investigators (22, 24). The *P. vivax* samples contained granular pigment located in the monocyte-lymphocyte layer that was not present in the other samples.

One blood sample from an asplenic patient contained numerous Howell-Jolly bodies. These erythrocyte inclusions also fluoresce brightly with acridine orange staining in the QBC tubes; however, they are larger than the parasite chromatin dots and do not contain adjacent cytoplasm, which assists in their differentiation from true intraerythrocytic organisms.

In this study, the QBC system showed 100% correlation with Giemsa-stained peripheral blood smears for the detection of parasitemia in patients with babesiosis. More importantly, the QBC allowed significantly more rapid detection of organisms in samples with low-grade parasitemia. Fresh samples appeared to be optimal for examination, and yet parasites could be reliably identified in all cases. The localization of *Babesia* organisms, characterized by large numbers of extracellular forms above the platelet layer and scattered intraerythrocytic forms, differed markedly from the malaria cases. It appears that the buoyant density of *B. microti*-parasitized erythrocytes was not appreciably altered. In the malaria samples, various morphologic forms as well as visible pigment were helpful differential features. Malaria infections that exhibit only rare ring forms may be difficult to distinguish from babesiosis both by smear and by QBC and require clinical correlation. Currently, exact malaria species identification with the QBC method alone is not optimal (1, 10).

The observation that large numbers of extracellular *Babesia* organisms concentrated above the platelet layer in the QBC tubes is intriguing given the risk of transfusion-transmitted disease. Jacoby and colleagues reported a severe case of babesiosis in an asplenic patient acquired from a platelet transfusion, postulated to be due to contamination of the small number of erythrocytes contained in the platelet product (12). Alternatively, our findings suggest that extracellular parasites potentially concentrate in the platelet fraction during blood component preparation from a parasitemic donor, thus providing a particularly large inoculum of parasites. We are unaware of other reports of platelet transfu-

TABLE 1. Comparison of peripheral smear and QBC results for 19 blood samples positive for babesiosis or malaria

Disease and organism	No. of samples	No. of patients	Peripheral smear		QBC	
			Morphology	Level of parasitemia (%)	Morphology	Localization[b]
Babesiosis						
Babesia microti	9	7	Ring (9/9) (tetrads [3/9])	0.02–0.1 (7/9); 0.5 (2/9)	Pyriform; rings; tetrads rare (1/9)	Extracellular, above plt layer (5/9)[c]; intracellular, scattered in RBC layer (9/9)
Malaria	10	6		<1 (10/10)	Rings (10/10)	Intracellular, upper RBC-granulocyte interface (10/10)
P. vivax	5	3	Rings, growing trophs, gametocytes		Trophs, gametocytes, rare schizonts	Pigment in lymphocyte-monocyte layer (5/5)
Plasmodium falciparum	4	2	Rings		Rings	Extracellular, above plt layer (2/4)
Plasmodium spp. (NOS)[d]	1	1	Rings		Rings	

[a] For some results, the ratio of samples with the given characteristic to total number of samples is given.
[b] plt, platelet; RBC, erythrocyte.
[c] Four of four fresh samples (examined within 24 h).
[d] NOS, not further specified.

sion-associated babesiosis, however, and this risk remains speculative.

In the appropriate clinical setting, QBC positivity may obviate the need for meticulous and time-consuming blood smear examination. However, since the capillary tubes are not permanent, smears should be made and kept as part of the permanent record and are necessary to determine the degree of parasitemia, or in those cases in which the diagnosis of malaria is also possible. A current limitation of QBC analysis is the cost of equipment (approximately $3,500) and special supplies ($2 to $3 per test).

The QBC method appears to be at least as sensitive as blood smear analysis for detecting *Babesia* organisms. We have not determined whether the QBC is a more sensitive method for parasite detection, as is the case with malaria. An important consideration, especially for a busy clinical laboratory, is that negative samples can be screened rapidly and that no false positives were found. In addition, the small and portable ParaLens adapter effectively eliminates the need for a separate conventional fluorescence microscope.

In summary, this report demonstrates the reliable identification of *Babesia* organisms in peripheral blood by the QBC technique. A larger prospective study is necessary to evaluate its diagnostic sensitivity and reproducibility. We anticipate that with more experience, QBC analysis will likely be a useful method for the detection of parasitemia in areas where *Babesia* spp. are endemic.

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