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Evaluation of a Real-Time PCR Assay Using the LightCycler System for Detection of Parvovirus B19 DNA

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We evaluated the artus RealArt Parvovirus B19 LC PCR reagent (artus biotech USA, San Francisco, Calif.) for real-time PCR detection of parvovirus B19 DNA by retesting 71 specimens previously submitted to our laboratory. The artus assay, which produces a quantitative result and provides an internal PCR control, appeared to be slightly more sensitive than our conventional qualitative PCR assay.

Human parvovirus B19 is a small-DNA virus of the family *Parvoviridae* that infects red blood cell precursors in the bone marrow. The virus is spread by the respiratory route, primarily during childhood, when it most commonly causes a febrile rash illness called erythema infectiosum, or fifth disease (2, 3). In the normal host, the disease is generally mild, with recovery being the rule. Infection of patients with underlying conditions may be more severe. Infected individuals with diseases shortening red blood cell life spans, such as sickle cell disease, may develop an aplastic crisis requiring red cell transfusions (9). Immunocompromised individuals who are unable to mount an antibody response to the virus can develop persistent infection with resulting chronic anemia (8). In these cases, administration of intravenous human immunoglobulin can reduce viremia and ameliorate the anemia (7, 11). B19 virus can also cross the placenta, resulting in fetal hydrops and spontaneous abortion (1, 5).

Laboratory diagnosis of parvovirus infection relies primarily upon serological methods to demonstrate an antibody response. In cases of individuals who cannot mount an antibody response, PCR-based methods to detect parvovirus DNA are used. For the past 10 years, our laboratory has offered a conventional PCR assay for detection of parvovirus DNA. Recently, a commercial assay utilizing real-time detection of parvovirus DNA using the LightCycler has become available. Because this assay offers the potential benefits of a quantitative result, an internal control of assay performance, labor savings, and decreased time to results, we carried out a comparison of this kit and our conventional PCR assay.

The artus RealArt Parvo B19 LC PCR assay, sold as an analyte-specific reagent, provides all the reagents needed to amplify a specific region of the parvovirus B19 genome and an internal control consisting of a second heterologous target. The internal control is detected in a different fluorimeter channel from the B19 target and can be used to identify the presence of PCR inhibitors or as a DNA isolation control. The artus assay was used to retest 71 specimens from 39 patients submitted to our laboratory over the past 3 years to rule out the presence of parvovirus B19 DNA. Specimens included 63 serum or plasma specimens, 4 cerebrospinal fluid (CSF) specimens, 2 bone marrow specimens, 1 amniotic fluid specimen, and 1 cord blood specimen. DNA was extracted at the time of submission by using either the manual QIAGEN Mini kit or the automated MagNA Pure method, using the total nucleic acid kit, and the extracts were stored at −70°C prior to retesting. The artus assay was used according to the manufacturer's instructions. Because the assay can be used to produce either quantitative or qualitative results, we tested some specimens qualitatively and others quantitatively. Briefly, 5 μl of extracted DNA was added to the appropriate LightCycler capillary containing reaction mix consisting of primers and probes specific for parvovirus B19 and the internal control, *Taq* enzyme, magnesium ions, nucleotide triphosphates, internal control target, and buffer. Each run contained at least one negative control consisting of 5 μl of water. For the assays run quantitatively, the full set of five standards served as the positive controls and results were expressed in international units (10) per milliliter of blood. For assays run qualitatively, a single positive control consisting of the 10-IU/μl standard was included.

For the conventional assay, testing had been performed previously using primers reported by Koch and Adler (6) that amplify a 699-bp fragment of the VP1 gene. Briefly, 10 μl of specimen was added to 90 μl of reaction mixture composed of

TABLE 1. Comparison of artus assay with conventional PCR for detection of parvovirus B19 DNA

Specimen	No. tested	SLCH[a] lab result	No. with Artus result[b]	
			POS	NEG
Serum/plasma[c]	47	Positive	46	1*
	17	Negative	2**	15
CSF	3	Positive	3	0
	1	Negative	0	1
Bone marrow	1	Positive	0	1*
	1	Negative	0	1
Amniotic fluid	1	Positive	1	0

[a] SLCH, St. Louis Children's Hospital.
[b] POS, positive; NEG, negative; *, negative when retested by the SLCH laboratory assay; **, no change in result when retested by both assays.
[c] Includes a single cord blood specimen.

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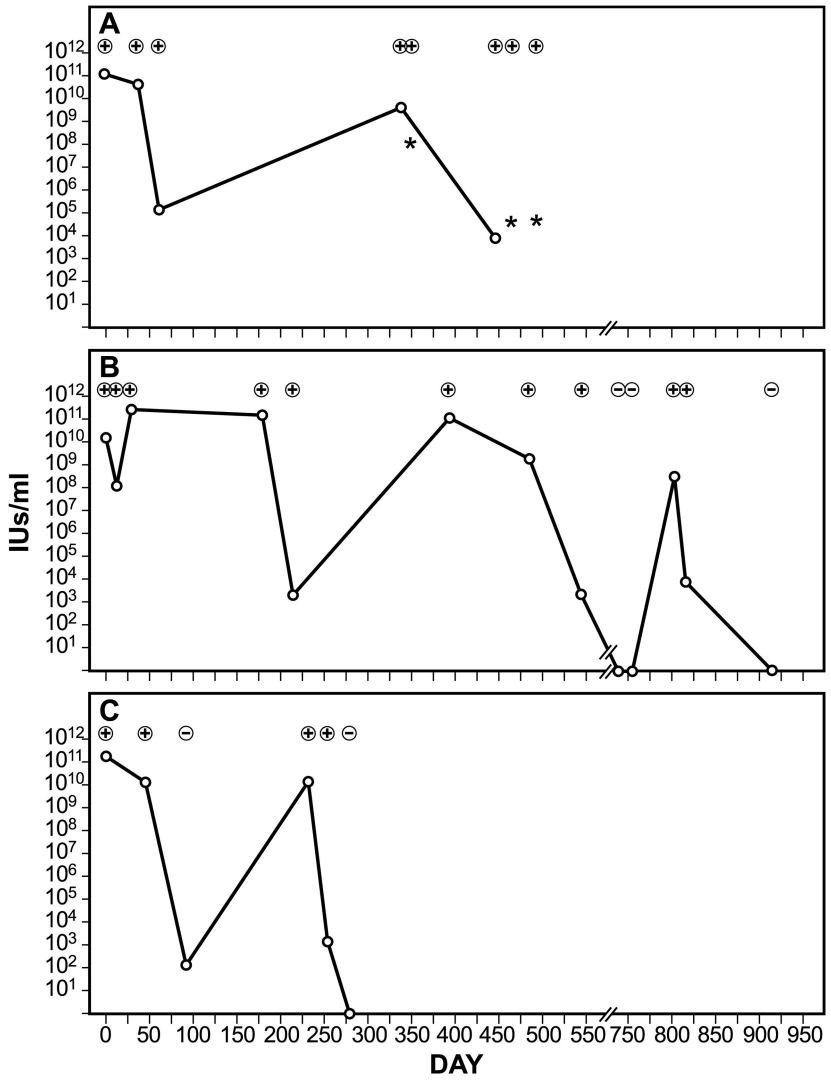

FIG. 1. Fluctuations in the levels of parvovirus B19 viremia with time in three patients with persistent parvovirus infections. Day 0 represents the first day that specimens were obtained for each patient. The level of viremia is expressed in international units per milliliter of blood as determined by the artus assay. The + and − symbols in each panel represent the conventional PCR qualitative result for each specimen. Each asterisk in panel A represents the quantitative level of B19 DNA in cerebrospinal fluid specimens obtained from that patient.

1× Stratagene Opti-Primer buffer no. 2, 100 pmol (each) primer, 200 μM (each) deoxynucleoside triphosphate, 0.2% dimethyl sulfoxide, and 2 U of Perkin-Elmer AmpliTaq *Taq* polymerase. Cycling was performed in a Perkin-Elmer 9600 thermocycler with the following program: 94°C for 3 min; then, 40 cycles of 94°C for 20 s; 50°C for 15 s; 72°C for 20 s; and last, 72°C for 2 min. Following cycling, 18 μl of product was analyzed using agarose gel electrophoresis.

The specimens included 52 reported previously as positive and 19 reported previously as negative. Testing with the artus assay produced results that agreed with the previous results in 67 (94%) cases (Table 1). All specimens testing negative for the presence of parvovirus DNA produced amplification with the internal control, indicating a lack of PCR inhibitors. The four discrepant specimens included two reported originally as positive and two reported as negative. To resolve the discrepancies, the four specimens were retested by both assays. For the two specimens initially negative by the conventional assay and positive by the artus test, retesting produced results identical to the initial results for both assays. One specimen produced an initial artus result of 134 IU/ml, suggesting that a low titer of virus was the reason for the negative results in the conventional assay. Likewise, the second discrepant specimen had a relatively low titer of 79,000 IU/ml and a negative conventional result, although seven other specimens with approximately equal or lower titers were positive in the conventional assay. Because both specimens were from patients with chronic parvovirus infections who had multiple positive specimens, it is likely that the artus results were true positives. Although false-positive results of the initial conventional PCR assay cannot be ruled out, for the remaining two discrepant specimens, retesting resulted in negative results by both assays for specimens originally reported as positive by the conventional assay. The lack of inhibition noted in these specimens suggested degra-

dation of a low titer of B19 DNA during storage. After retesting, the artus assay agreed with the conventional PCR for 69 (97%) of specimens.

The 39 positive specimens tested quantitatively yielded titers from 1.3×10^2 to 4.4×10^{11} IU/ml of original specimen. Although in most cases a qualitative parvovirus PCR result is sufficient, with chronic infection it may be desirable to produce a quantitative result to assess responses to the administration of human immunoglobulin. Quantitative results from multiple specimens obtained over time on three separate patients with chronic parvovirus infections are shown in Fig. 1. The apparent changes in viral titers noted in these specimens likely represent responses to the administration of human immunoglobulin, although we could not confirm this because specimens had been stripped of identifiers before testing.

In addition to blood specimens, we also tested an amniotic fluid specimen and four CSF specimens. The amniotic fluid and the companion blood specimen from the same patient were both positive, confirming infection of the fetus, although the amniotic fluid contained a titer of virus close to 4 logs lower than that of the blood. Interestingly, one of the patients with chronic parvovirus infection also had CSF specimens submitted that were positive for the presence of parvovirus B19 DNA (Fig. 1A). Neurological complications have been associated with parvovirus B19 infections, but the mechanism of these effects is unclear. In some cases, B19 DNA has been found in the CSF of patients with encephalitis or meningitis (4).

In summary, the artus assay was simple to use, provided rapid results, included an internal control of the PCR, and gave the option of producing quantitative results. Our results indicated that the artus assay was more sensitive than the conventional assay we now use and was able to accurately detect B19 DNA in a variety of specimens. The inclusion of an amplification control was particularly useful for ruling out PCR inhibitors and would presumably help satisfy laboratory regulatory requirements for amplification controls. For laboratories using the LightCycler, the artus parvovirus assay represents a useful option for detection of parvovirus DNA.

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