

Identification and Characterization of Parvovirus B19 from Chilean Clinical Specimens

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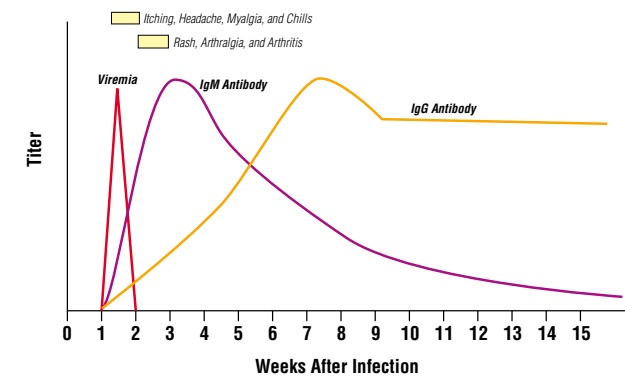
OBJECTIVES

- Identify Parvovirus B19 infections in patients from the Santiago Metropolitan Area showing clinical symptoms/manifestations for Parvovirus B19.
- Perform PCR amplification, cloning and DNA sequence determinations for nucleotide and amino acid sequence variability studies.
- Develop a method to quantitate Parvovirus B19 using Taqman Technology.

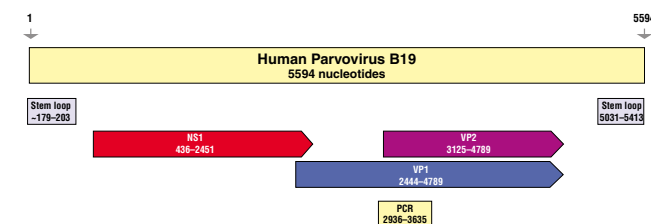
INTRODUCTION

- Parvovirus B19 is a small 22-nm icosahedral nonenveloped virus of the Picornaviridae family.
- The Parvovirus B19 genome consists of a linear single-stranded DNA molecule of ~5,600 nucleotides.
- Clinical manifestations of Parvovirus B19 infections include Erythema infectiosum (EI) or fifth disease in childhood, transient aplastic crisis (TAC) in patients with hemolytic disorders, neonatal death in pregnant women, acute arthropathy in adults and persistent anemia in immuno-compromised patients.
- The absence of a lipid envelope and limited DNA content make Parvovirus B19 very resistant to physicochemical inactivation.
- Transmission of Parvovirus B19 through the administration of plasma products is well documented.

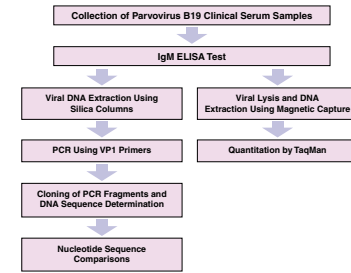
Natural History of Parvovirus B19 Infection



Human Parvovirus B19 ssDNA



METHODS



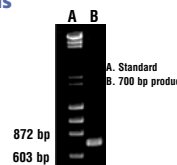
RESULTS

Clinical and IgM ELISA Data From Chilean Samples

Sample ID	DIAGNOSIS	IgM PV-B19
SCL1	Erythema infectiosum	Positive
SCL2	Arthralgia	Negative
SCL3	Pancytopenia	Negative
SCL4	Immunodeficiency	Negative
SCL5	Liver inflammation	Negative
SCL6	Purpura Schonlein-Henoch	Negative
SCL7	Febrile syndrome	Negative
SCL8	Not Disclosed	Negative
SCL9	Vasculitis	Positive
SCL10	Hydrops	Positive
SCL11	Hydrops	Positive
SCL12	Hydrops	Negative
SCL13	Purpura Schonlein-Henoch	Negative
SCL14	Hydrops	Positive
SCL15	Purpura Schonlein-Henoch	Positive
SCL16	Purpura Schonlein-Henoch	Positive
SCL17	Hemolytic anemia	Positive
SCL18	Exanthem	Positive
SCL19	Not Disclosed	Positive
SCL20	Not Disclosed	Positive
SCL21	HIV/AIDS	Positive
SCL22	Not Disclosed	Positive

PCR of Parvovirus B19 DNA for Sequence Analysis

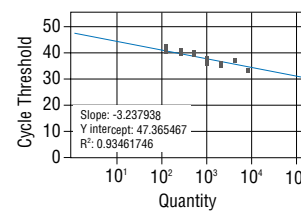
Primer	Size	Sequence	Region	Product Size
SN-K1	19	ATAAATCCATATACTCATT	VP1	700
SN-K2	18	CTAAAGTATCCTGACCTT	VP1	700



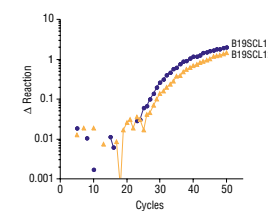
Quantitation by TaqMan PCR

Internal Chiron ID	Volume Tested (µL)	Titer (IU/mL)
B19SCL1	250	515,215.50
B19SCL12	50	62,422.96

Standard Curve Using CBER Parvovirus B19 Panel



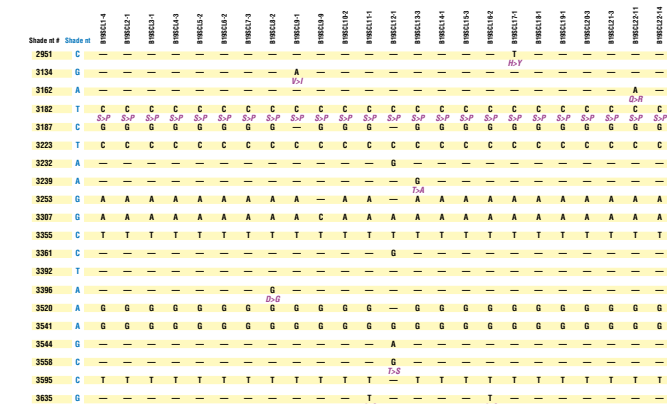
Real Time PCR



Nucleotide and Amino Acid Sequence Comparison Between the 700 bp Fragments and the Corresponding Region Reported by Shade et al. (J. Virol. 58: 921-936, 1986)

Clinical Sample	Nucleotide Substitutions	Homology (%)	Amino Acid Changes	Identity (%)
SCL1-4	9	98.7	1	99.6
SCL2-1	9	98.7	1	99.6
SCL3-1	9	98.7	1	99.6
SCL4-3	9	98.7	1	99.6
SCL5-2	9	98.7	1	99.6
SCL6-2	9	98.7	1	99.6
SCL7-3	9	98.7	1	99.6
SCL8-2	10	98.6	1	99.6
SCL9-1(*)	8	98.9	2	99.1
SCL9-9(*)	9	98.7	1	99.6
SCL10-2	9	98.7	1	99.6
SCL11-1	10	98.6	2	99.1
SCL12-1	9	98.7	2	99.1
SCL13-3	11	98.4	2	99.1
SCL14-1	9	98.7	1	99.6
SCL15-3	9	98.7	1	99.6
SCL16-2	10	98.6	2	99.1
SCL17-1	10	98.6	2	99.1
SCL18-1	9	98.7	1	99.6
SCL19-1	9	98.7	1	99.6
SCL20-3	9	98.7	1	99.6
SCL21-3	9	98.7	1	99.6
SCL22-11(*)	10	98.6	2	99.1
SCL22-14(*)	10	98.6	1	99.6

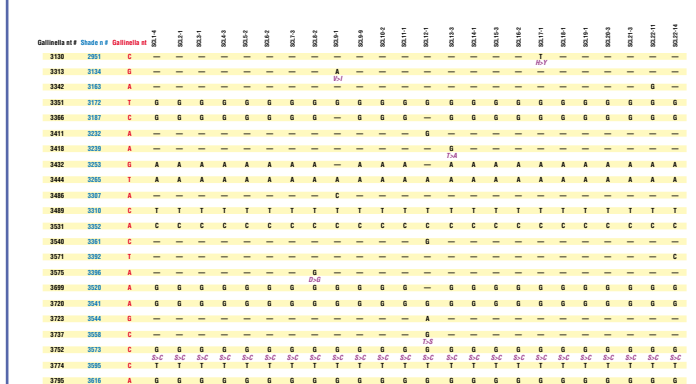
(*) Samples infected with multiple isolates.



Nucleotide and Amino Acid Sequence Comparison Between the 700 bp Fragments and the Corresponding Region Reported by Gallinella et al. (Genbank Accession AF162273)

Clinical Sample	Nucleotide Substitutions	Homology (%)	Amino Acid Changes	Identity (%)
SCL1-4	11	98.4	1	99.6
SCL2-1	11	98.4	1	99.6
SCL3-1	11	98.4	1	99.6
SCL4-3	11	98.4	1	99.6
SCL5-2	11	98.4	1	99.6
SCL6-2	11	98.4	1	99.6
SCL7-3	11	98.4	1	99.6
SCL8-2	12	98.3	2	99.1
SCL9-1(*)	11	98.4	2	99.1
SCL9-9(*)	11	98.4	1	99.6
SCL10-2	11	98.4	1	99.6
SCL11-1	11	98.4	1	99.6
SCL12-1	11	98.4	2	99.1
SCL13-3	12	98.3	2	99.1
SCL14-1	11	98.4	1	99.6
SCL15-3	11	98.4	1	99.6
SCL16-2	11	98.4	1	99.6
SCL17-1	12	98.3	2	99.1
SCL18-1	11	98.4	1	99.6
SCL19-1	11	98.4	1	99.6
SCL20-3	11	98.4	1	99.6
SCL21-3	11	98.4	1	99.6
SCL22-11(*)	12	98.3	1	99.6
SCL22-14(*)	12	98.3	1	99.6

(*) Samples infected with multiple isolates.



SUMMARY AND CONCLUSIONS

- Twenty two serum specimens from patients of the Santiago Metropolitan Area showing clinical symptoms & manifestations for Parvovirus B19 infections were investigated using anti Parvovirus B19 IgM ELISA, PCR, cloning and DNA sequencing.
- PCR amplification allowed detection of Parvovirus B19 serum samples that were IgM ELISA negative.
- Amplification, cloning and nucleotide sequence determination of a 700bp VP1 fragment derived from the different clinical samples enabled to perform nucleotide and amino acid sequence comparisons between the different isolates and those reported by Shade et al.(1986) and Gallinella et al. (1999).
- Nucleotide sequence homologies of 98.3 – 98.9% and amino acid sequence identities of 99.1 – 99.6% were obtained when the sequences determined for the Chilean samples were compared with the corresponding sequences reported by Shade and Gallinella, respectively.
- Nucleotide sequence determination of independent clones derived from the same serum sample revealed that two sera were infected with more than one Parvovirus B19 isolate (quasispecies).
- Quantitation by TaqMan of two clinical samples from patients with erythema infectiosum and hydrops showed Parvovirus B19 titers of 64,422 IU/mL and 515,215 IU/mL, respectively.