EXPRESSION AND PURIFICATION OF INOSINE MONOPHOSPHATE DEHYDROGENASE (IMPDH) FROM *PNEUMOCYSTIS CARINII*

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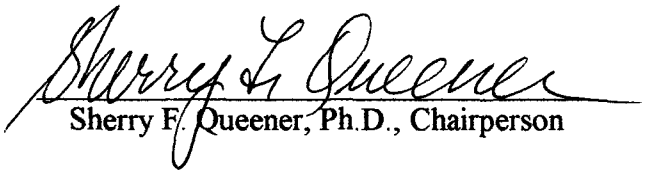

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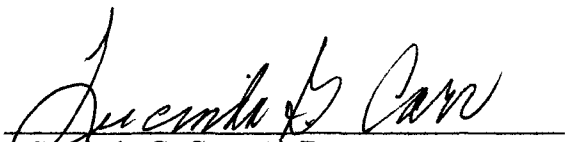

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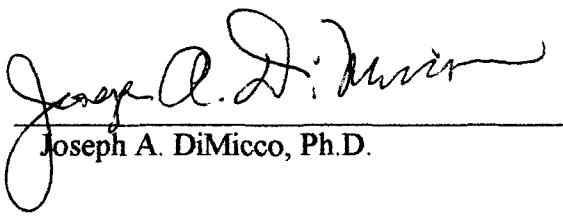

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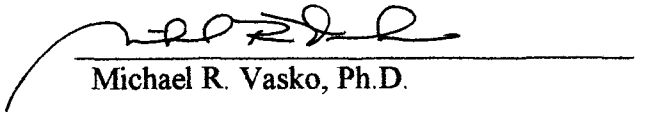

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Abstract

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Expression And Purification Of Inosine Monophosphate Dehydrogenase (IMPDH) From *Pneumocystis Carinii*

Pneumocystis carinii is an opportunistic pathogen that affects immune compromised individuals, including those receiving cancer chemotherapy, those on immunosuppressive therapy and individuals with AIDS. Previous studies have suggested that inosine monophosphate dehydrogenase (IMPDH) may be a novel drug target in *P. carinii*. The objective of this research was to identify and characterize the *P. carinii* IMPDH protein. With this goal in mind, attempts were made to purify both the native form of *P. carinii* IMPDH and a recombinant form of the enzyme. The recombinant form of the protein was examined by expression of recombinant *P. carinii* IMPDH in two different bacteria. Recombinant *P. carinii* IMPDH was first expressed in IMPDH-deficient Sϕ1101 *E. coli* using a pET expression vector. Purification of the recombinant protein from this expression system employed G-100 size exclusion chromatography and IMP-affinity chromatography. These experiments revealed that column chromatography allowed for the purification of IMPDH, but the Sϕ1101 bacteria were producing catalytically active IMPDH that interfered with the isolation of the recombinant *P. carinii* IMPDH protein. Recombinant *P. carinii* IMPDH was also expressed in IMPDH-deficient LH3 *E. coli* using a pTactac expression vector. Immunologically reactive soluble recombinant *P. carinii* IMPDH was produced in this expression systems, but the bacteria were not producing enough soluble recombinant protein to support extensive biochemical analysis or column purification. The inability to obtain extensive data using

the recombinant form of the enzyme led to the search for the native IMPDH protein from *P. carinii.* Investigation of the native protein was undertaken with proteins isolated directly from the *P. carinii* organism. A two-column purification strategy utilizing G-100 gel filtration chromatography and IMP-affinity chromatography was devised and executed using a crude extract of proteins from *P. carinii.* The results suggested that this crude extract of proteins from *P. carinii* was contaminated with rat mitochondrial proteins that interfered with the isolation of native *P. carinii* IMPDH. To further investigate *P. carinii* IMPDH, better isolation methods for *P. carinii* as well as other expression vectors and expression systems should be explored.

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