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To: The Moran Foundation Scientific Advisory Committee
From: GJ Buffone, Ph D
Re: Progress Report
Date: November 17,1986

Project title: Measurement of virus by DNA hybridization analysis: Sample preparation and non-isotopic labels (1-86-0018)

We have evaluated the use of hydroxyapatite chromatography columns for use in cleaning up urine samples prior to analysis with DNA probes labeled with non-isotopic indicators. Although it seems this technology would provide approiately prepare the sample for analysis, the time and trouble involved would preclude the use of this approach in a routine laboratory setting.

We have also evaluated the use of a significantly shorter extraction procedure, which eliminates the use of the ultracentrifuge, phenol and the time required for digestion of the associated protein. Although recovery of virus was good, the same problems encountered with direct urine analysis were encountered. Namely, aberrant color development on the filter.

As such we have decided to use a new strategy that would eliminate the use of nitrocellulose filters and hence the problem of aberrant color development that seems to be inherent in such a system when semipurified DNA is used. We are now cloning small segements of the CMV genome into M13 a bacteriophage vector which will allow us to generate single stranded probes. This newly cloned probe along with the probes in pBR322 will allow to set up either a sandwich or strand displacement assay. Either of which will allow us to avoid the need for extensive sample clean up.

Once available the assay will be evaluated for its applicability to screening large neonatal populations for CMV infections.