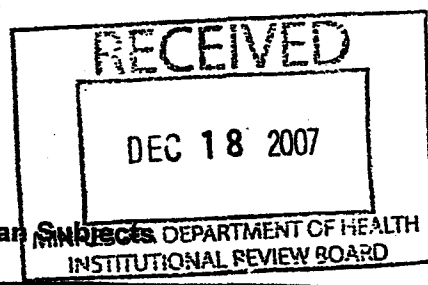


Minnesota Department of Health
Institutional Review Board



Application for Approval of Research with Human Subjects

A. Description of the subject population - number of subjects, age range, how subjects will be identified and selected;

Study Design:

The Minnesota statute 145.898 which mandated the Minnesota Department of Health (MDH) to develop Infant Death Investigation Guidelines to facilitate the uniform investigation and determination of causes of death for sudden, unexplained infant death helped in establishing an existing data base of all infants who died of SIDS in the state of Minnesota. Using this data base, which is kept at the MDH Center of Health Statistics, we will obtain the NBS of infants who died of SIDS for molecular testing for congenital CMV infection [figure 1]. Our collaborators at the MDH Newborn Screening Program, who have an existing partnership with the MDH Center for Health Statistics, will identify and retrieve the archived NBS of all infants who were born in Minnesota and died of SIDS in the state of Minnesota between 1997 and 2006 [see attached letter of support from Mr. Mark McCann]. The study group will consist of infants who died of SIDS whose NBS will be retrieved for molecular analysis. The control group will be a cohort of randomly selected, anonymized, matched for age and gender, newborn babies, whose NBS will also be retrieved. We expect approximately 250 NBS at each group. Prior to transfer to our research laboratory, all NBS will be assigned a random identification key, known only to the MDH Newborn Screen Program. At the time of DNA extraction and amplification we will be blinded as to the identity and status, (SIDS versus control), of each blood spot.

For PCR analyses, six 3 mm punches will be obtained from each NBS for DNA extraction. DNA will be extracted using the X-tractor Gene Solid Sample Reagent Pack (Sigma). Nucleic acid extraction will be performed according methods established for the Universal Solid Tissue Sample DNA Extraction CorProtocol 14202 using the Corbett X-tractor Gene. Final elution volume will be 100 µl. Extracted DNA elution will be the template for a nested PCR reaction using designated primers targeting the CMV *UL144* gene, a novel tumor necrosis factor receptor homolog. Positive PCR product will be gel-purified using Qiagen extraction kit and than PCR products will be cloned into the vector pCR 2.1 (Invitrogen®). Following completion of molecular analyses of all NBS we will be unblinded as to the status of each NBS and assign each blood spot to the study versus the control group. We will than compare the rate of positive CMV DNA between the two groups as our study end-point.

Identity of all subjects (study and control groups) will not be revealed to the researchers or anyone else outside the MDH at any point of the study and will remain anonymous!

B. Explanation of subject involvement in the research (the who, what, when, and how of subject involvement);

The subjects themselves will have no involvement in the study, as the study is designed to analyze their anonymized NBS, which are archived at the MDH. Subjects will not be contacted.

C. Summary of data analysis or statistical methods to be used in the study;

The prevalence of CMV genome found in the NBS of the study group will be compared to that found in the control group.

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