INFECTIVITY STUDIES

BIOVIR IN-HOUSE CAPABILITIES

Cryptosporidium

- *Animal Model*
- *Cell Culture*
- *Excystation*
- *Vital Dyes*

Giardia

- *Animal Model (G. muris)*
- *Excystation*
- *Vital Dyes*

Studies performed under documented Good Laboratory Conditions. In-house capabilities eliminate need for inter-laboratory transfer of in-process samples.

INTRODUCTION

Current issues associated with drinking water treatment, waste water treatment, and wastewater reuse have highlighted the importance of determining treatment system efficacy in inactivating Cryptosporidium and Giardia.

When evaluating disinfection efficacy, data is generally produced at bench or pilot scale. Large scale challenge studies are difficult because of the lack of availability of cysts or oocysts and/or the expense in producing numbers greater than 10^7 or 10^8 total organisms.

The current methods for determining Cryptosporidium oocyst and Giardia cyst viability and / or infectivity (see following discussion) each have their advantages and disadvantages from a scientific perspective. Issues with respect to viability versus infectivity for disinfection studies are currently being discussed within the scientific community. Until agreement is reached as to the "best" method, the method or methods which are ultimately employed in a disinfection study will be guided as much by regulatory requirements, or lack thereof, budgetary constraints, and time constraints as by scientific validity.

If a study is required to address regulatory issues, BioVir always recommends that the applicable regulatory body be involved in the planning stages in order to avoid completion of a project that may not satisfy the regulator.

ISSUES OF VIABILITY

Animal Infectivity

Cryptosporidium infectivity studies are normally conducted in mice. This has been considered the most conclusive method regarding *infectivity* of the organism because the gut of the mice are examined for evidence of infection. However, it is expensive and labor intensive requiring many neo-natal mice. Few environmental laboratories have the technical expertise to offer this service. BioVir is the only commercial laboratory in the U.S. which can perform complete crypto infectivity studies in-house.

Giardia infectivity studies are performed both in mice and gerbils with *Giardia muris* studies being performed in adult mice and *Giardia lamblia* studies performed in gerbils. Animal studies for giardia infectivity cost significantly less that those for cryptosporidium.

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Cell Culture

Cryptosporidium cultivation in a cell culture system (generally, HCT-8 cells) is now accepted. Recent research has shown favorable results in comparison with animal infectivity studies. Advantages over the animal system is that it is more sensitive (ie. shows infectivity more readily) and is considerably less expensive. Disadvantages are that it may not reflect true infectivity relative to a whole animal.

Giardia has not yet been cultured in a cell system.

Excystation

During laboratory induced excystation, the cysts or oocysts are exposed to an environment similar to that of a host gastrointestinal system. Therefore, excystation is a measure of the ability of the organism to react to chemical changes in it's immediate environment. Since the sporozoites within the oocyst are the only living things (not the oocyst itself) excystation really measures the intact physical and biochemical nature of the oocyst and may not measure the infectivity of the sporozoites. Similarly, giardia cysts which produce trophozoites in-vitro, demonstrates the cyst's intact physical and biochemical nature, however the trophozoites' ability to infect is not proven.

In excystation procedures using either giardia or cryptosporidium, positive results evoke discussions regarding *viability* versus *infectivity*. However, if a very conservative approach is taken, one could argue that if excystation occurs infectivity can be assumed. Costs associated with this testing are quite reasonable when compared to the Animal Infectivity or Cell Culture methods indicated above.

"Viability" Stain

Vital stains are chemicals which interact, or not, with cells, such as cysts or oocysts, in such a manner as to indicate viability of the organism in question. In the case of cryptosporidium oocysts and giardia cysts a combination of 4'6-diamidino-2-phenylindole (DAPI) and Propidium Iodide (PI) is commonly used. They both fluoresce, PI is excluded by live organisms and DAPI is absorbed by intact DNA. Similar to Excystation this is not a 100% test but use of this method is growing. This method is the least costly to employ of the methods described here.

Vital stains used in conjunction with excystation and DIC/Hoffman microscopy will reveal if the nuclei of these organisms are present, contain DNA and that, if placed into the right environment, will excyst and release cryptosporidium sporozoites or giardia trophozoites. Again, a very conservative interpretation of positive results would be that the viable organisms are infective.