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USES OF PCR IN ENVIRONMENTAL MOLD INVESTIGATIONS

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PCR stands for polymerase chain reaction. DNA polymerase is an enzyme extracted from a thermophilic bacterium. The polymerase reaction replicates specific DNA fragments to detectable concentrations. A few terms need to be defined: A primer is a DNA fragment that exactly matches that of the organism of interest. The template is the DNA from the unknown sample. Amplification occurs if any of the DNA in the unknown sample matches exactly the DNA from the primer. If so, the polymerase causes the sample DNA to be replicated (the concentration will be increased). Concentration curves can be established by adding known DNA to the unknown sample. This method is extremely specific and sensitive and can detect DNA from a single spore.
For fungi, primer fragments may represent a group of fungi (e.g., Penicillium and Aspergillus), a single genus (e.g., Aspergillus), a single species (e.g., Aspergillus fumigatus) or a strain (one of the many genetic variations that occur in each species). While sensitive and precise, PCR is strongly affected by contaminants in the solutions that are part of the assay. Both dust and air contain contaminants that can interfere with PCR and controls need to be carefully designed to ensure that such problems do not occur (McDevitt et al., 2007).

USE OF PCR IN ENVIRONMENTAL INVESTIGATIONS

1. Tracing infectious agents to a source. The primer is a fragment of the infectious agent cultured from the host. Templates are DNA fragments extracted from air or from environmental reservoirs. Amplification occurs if the fragments match exactly. Strain-specific primers must be used if the organisms are common in the environment.

2. Air monitoring for specific organisms of interest. The primers are fragments of DNA from known samples of the organisms of interest. The templates are DNA extracted from the air sample. Again, if amplification occurs, then the organism is present in the air sample. If appropriate controls are used, one can estimate how much of the organism is present. The incidence of *Aspergillus terreus* in a hospital has been tracked over time using PCR [Lignell et al., 2008]. You must know which fungi you are looking for and you must have a separate primer for each. Organisms not represented by a primer are not detected (i.e., they remain invisible to the test). Also, while you can gain quantitative data from this test, it may be difficult to interpret until a large data base using the method has been accumulated. It can be readily used epidemiologically where each data point is compared to a paired health outcome.

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3. Another interesting environmental use of PCR is to study ecology of specific reservoirs. PCR methods are commonly used in water quality studies and for evaluation of biofilms. For fungi, one could choose a reservoir (e.g., house dust) and select a panel of fungi to study. Environmental data could be collected (say relative humidity, temperature, season, moisture content of dust, etc.). Then the test is run and the data compared to these variables. This could be done repeatedly on one dust sample while controlling the variables in the laboratory, or on many field samples where the variables of interest extend over an appropriate range.

ERMI

This is the Environmental Relative Moldiness Index developed by the USEPA. It uses 36 primers: 26 isolated from "water damage" fungi and 10 from "normal" indoor fungi. Templates are the DNA fragments isolated from dust or air samples. Using the results from this panel, an index of relative moldiness is calculated.

ERMI tells you the relative amounts of the listed fungi in the dust sample. A comparison can be made to a small data set from "moldy" and "randomly" selected homes. The index provides a continuous variable for use in epidemiological studies. This test might be useful for studying ecology of reservoirs as discussed above by evaluating the actual data from the test rather than the index.

The problems with ERMI as an environmental test are many. First, the panel of fungi used is open to serious question. It is not clear how the fungi were chosen, and many mycologists and aerobiologists would disagree with the list. A newer method has been published that uses only 12 fungi, but still, the list is open to question. (Vesper et al 2009) It is especially important to remember that the index is based on a very small series of epidemiological studies. (Vesper et al., 2008) This means that while there might be a correlation between water damage or health effects and the index, the correlation is suspect when tested on such a small data set, and even if it were relatively strong, one cannot depend on a relationship with either water damage or health outcomes in any single sample. Also, the calculations for the index have come into serious question (see AIHCE 2008).

ERMI DOES NOT TELL YOU:

1. Variability within a building.
2. Whether or not a building has a water problem.
3. The extent of the water problem.
4. Where the problem is.
5. Whether or not there are unusual fungi present.
6. Exposure if air samples are not used.

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Epidemiologists generally do not ask for perfect exposure measures. Measurement errors are compensated for by large numbers of samples. The ERMI index could be used in this type of investigation, however it would certainly benefit from further development. It is not useful at this time for incident investigations.

For environmental investigations PCR is best used to track specific organisms. Environmental infectious agents such as *Aspergillus fumigatus* or *Cryptococcus neoformans* can be tracked to a source. If infections have occurred, then strain matching may lead to the specific reservoir that led to exposure and disease. Samples may be bulk samples or air samples compared to cultured strains from the patients. Other specific fungi of interest could also be tracked, and the data correlated epidemiologically to specific health outcomes. PCR is also commonly used to evaluate the changing ecology of specific reservoirs. Bacterial biofilms are commonly studied using PCR. The biofilm is processed and its DNA is amplified. The patterns of DNA fragments recovered are compared to databases and over time.

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