

BioCop Women in Science Fellowship Report

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A. Initial Aims

1. Overall aim is to transfer technology of transcriptomic analysis methods and understand the different extractions methods employed by the host lab.
2. The work plan is two fold. Over a 14-day period the fellow will introduce cell culture techniques, RNA extraction, cDNA synthesis, and quantitative RT-PCR analysis while learning the methods of extraction and clean up of various types of grain samples.
3. The expected outcomes/benefits should result in a more fluid analysis process from sample extraction to transcript analysis that can be carried out at all of the partner laboratories in WP's 1 and 10.

Technologies in which training is being requested:

-Oats, grains, baby food, maize, and breakfast cereal extraction/analysis.

-Clean-up methods using MycoSep columns and possibly immunoaffinity columns.

Revised Aims

As per my correspondence with Dr. Chen Situ, BioCop Gender Manager, the following was discussed:

Transfer technology on my side will not be as extensive. I will contribute to between one to two introductory lectures, introduce the protocol, and discuss the needed laboratory arrangements required to begin the bioassay in the host's lab.

B. Determination of Trichothecenes in Duplicate Diets (Summary of Results)

Extraction, clean up, dilution, and measurement of contaminated and non-contaminated matrices are standard steps when determining the presence and amount of trichothecene mycotoxins. While extraction methods are generally the same using most commonly an Acetonitrile/Water solution or a Methanol/Water solution, clean up and measurement can vary depending on the preference of the analyst and the type of laboratory (chemical and/or biological).

Using wheat contaminated and blank breakfast cereals, it was determined that most of the clean-up methods were sufficient for detection of a wide range of trichothecenes, measured by GC-MS. In our experiments, clean-up methods varied from simple one-step MycoSep columns without dilution to MycoSep multi-step columns, BondElut columns, and Immunoaffinity chromatography (IAC). IAC was specific for HT2 and T-2 toxin whereas the other columns were thought to elute Type A and Type B trichothecenes, and other *Fusarium* toxins. The chromatographs in the supplemental data show the accuracy of the completed experiments as measured by GC-MS when compared to control samples. The combination of the MycoSep 227/Multistep 216 columns produced an excellent clean up with minimal amount of background interference compared to BondElut and IAC alone. Only with multiple dilution steps did the BondElut clean-up method produce similar results.

After evaluating these results it was decided that the fellow would produce samples to be used in the subsequent experiments after the fellowship to determine if the new BioCop bioassay method could measure similar amounts of trichothecenes. Studies are currently ongoing.

C. Statement on Fellowship Programme

Exposure to residue analysis via GC-MS has given me a new found appreciation for the role that analytical chemists play in developing extraction, cleanup, and measurement validation techniques with respect to food contaminants. The interaction between workpackages has taken on a new meaning with regards to better clarity of what both sides, chemical and biological, can contribute.

This fellowship should definitely be continued to encourage participation of women scientists in all disciplines.

I wish to thank the group led by Ir Hans van Egmond at the National Institute for Health and the Environment in Bilthoven, and Ad Jekel for his direct supervision and guidance during my fellowship.

Most importantly, I would like to thank Dr. Chen Situ for her leadership role in the Biocop Women in Science Fellowship board and BioCop's TMG for awarding me with this fellowship to carry out the activities.