D8 Dating Spores With the Carbon-14 Bomb Pulse

Bruce A. Buchholz, PhD, Lawrence Livermore National Laboratory, Center for Accelerator Mass Spectrometry, Mail Stop L-397, 7000 East Avenue, Livermore, CA 94550; Jenny A. Cappuccio, PhD, Lawrence Livermore National Laboratory, Biosciences Division, 7000 East Avenue, Livermore, CA 94551; and Miranda J. Sarachine, PhD, Lawrence Livermore National Laboratory, Center for Accelerator Mass Spectrometry, Mail Stop L-397, 7000 East Avenue, Livemore, CA 94551*

After attending this presentation, attendees will understand how biological materials produced in the past 55 years can be dated using the carbon-14 (^{14}C) bomb-pulse. Attendees will learn how the carbon-14 (^{14}C) content of biomolecules serves as a chronometer of synthesis between 1955 and the present.

The presentation will impact the forensic science community by showing how a recently produced bioagent can be distinguished from one drawn from a historical archive.

Investigators of bioagent incidents or interdicted materials need validated, independent analytical methods that will allow them to distinguish a recently made bioagent sample from material drawn from the archives of a historical program. Accelerator mass spectrometry (AMS) precisely measures $^{14}C/C$ concentrations in biological materials and has been used to date the synthesis of biomaterials over the bomb pulse era (1955 to present), fulfilling the law enforcement need to place bioagents in a chronological context.

Atmospheric testing of nuclear weapons during the 1950s and early 1960s doubled the concentration of ^{14}C in the atmosphere. After cessation of atmospheric tests in 1963, the level of $^{14}CO_2$ has decreased with a mean life of about 16 years, not due to radioactive decay, but due to mixing with large marine and terrestrial carbon reservoirs. The temporal variations of artificially high levels of atmospheric ^{14}C have been captured in organic material world-wide and thus offer an opportunity to determine a date of synthesis for biomolecules. Since ^{14}C is incorporated into all living things, this bomb-pulse is an isotopic chronometer of the past 55 years. The enhanced level of ^{14}C worked its way up the food chain from CO_2 so that all living things were labeled with the pulse.

The concentration of $^{14}C/C$ was measured in a variety of media, *bacillus* spores, and separated proteins from *bacillus* spores. Bacteria convert the carbon in their food sources into the biomolecules they need, just like plants and animals. The ^{14}C concentration of *Bacillus* spores reflects the radiocarbon content of the media in which they were grown. The incorporation of the food source isotopic signature occurs if the media is primarily carbohydrate (e.g., high glucose), primarily protein derived (excess nitrogen), or a blend. In a survey of commercial media we found that the ^{14}C concentration indicated that carbon sources for the media were alive within about a year of the date of manufacture and of terrestrial origin. Hence, bacteria and their products can be dated using their ^{14}C signature.

Bacillus spore samples (BSL1, biosafety level 1) were obtained from the LLNL archive as well as generated on site. The standards were *B. thuringiensis kenyae* spores (Bt ken, control spores) generated onsite with defined media and carbon free purification; The test samples include *B. thuringiensis israelensis (*Bti*), B. globigii* (Bg), and *B. thuringiensis kurstaki* (Btk) from the LLNL archive. The archive spores were produced and purified by means unknown to the researcher performing the extraction, in order to mimic real world specimens. The archived spores were contaminated with petroleum-derived carbon from solvents and detergents used during processing. Using a mechanical lyser and a variety of washes with carbon free KOH, HCl, and HOOH, contaminant carbon was removed from soluble proteins. Samples were dried and combusted to CO_2. The evolved CO_2 was purified, trapped, and reduced to graphite in the presence of iron catalyst in individual reactors. Graphite targets were measured for ^{14}C content by accelerator mass spectrometry.

Soluble proteins were purified sufficiently for accurate ^{14}C bomb- pulse dating. The insoluble fractions could not be cleaned using our procedures. Since media is contemporary, ^{14}C bomb-pulse dating of isolated soluble proteins can be used to distinguish between historical archives of bioagents and those recently produced.

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory.

Bomb Pulse Dating, *Bacillus* Spores, Protein

 ** Presenting Author*