1. Executive Summary

1.1.   How Research Added to Understanding

The most challenging technical goal of Phase I was to detect the presence of Ge_{4+} ions at the nano-molar concentration levels established by the FDA as the threshold for contamination. To achieve this goal we used electrophoretic-based techniques using electric fields to move ions through a channel and detecting their characteristic arrival time near the end of the channel. Unlike the traditional gel electrophoresis typically used for clinical chemistry (e.g., Western blot), we chose a capillary zone electrophoresis (CZE) method that would be more readily transferable to microfluidic chip fabrication processes.

1.2.   Technical Effectiveness

The project was highly successful technically. To our knowledge, the conductivity at infinite dilution for germanium had never been previously measured, and we have submitted this datum for scientific publication to the Journal of Nuclear Medicine. Upon testing with the standardized GeO_2-derived solutions, the miniature CZE system was able to detect the presence of Ge_{4+} ions in HCl solution at remarkably low (i.e., nM) concentrations with high repeatability.

1.3   Benefits to the Public

The low-cost proprietary assay system which was demonstrated in the Phase I project, could be incorporated into commercial generators or sold by third-party suppliers directly to hospitals. If commercialized, it would stimulate development of cost-effective generator-produced radiotracers in the USA, as has occurred in Europe. The employment of industry-standard microfluidics is a good match to systems currently being implemented for radiotracer synthesis, and would therefore likely to be adopted rapidly. Unfortunately the project was not selected for Phase II funding, and so commercialization of the technology may not be forthcoming.

2. Accomplishments of the Project

The technical goal of Phase I was to detect the presence of Ge^{4+} ions at the nanomolar concentration levels established by the FDA as the threshold for contamination. To achieve this goal we used electrophoretic-based techniques: using electric fields to move ions through a channel and detecting their characteristic arrival time near the end of the channel. Unlike the traditional gel electrophoresis typically used for clinical chemistry (e.g., Western blot), we chose a capillary zone electrophoresis (CZE) method that would be more readily transferable to microfluidic chip fabrication processes. As discussed above (Figs. 1,2), having a disposable microfluidic chip confers strong advantages to nuclear medicine users, since the radioactive chip can be removed from the reader system after the assay is over, thereby allowing additional studies to be performed using the reader system without delay.

3. Summary of the Project Activities

We accomplished our Phase I measurement goal using the small form-factor CZE apparatus shown in Fig. 1. The apparatus consisted of two solution wells connected through a 30-micron-diameter glass capillary, a 12 kV high voltage power supply connected to a platinum electrode in each of the wells, and a capacitatively-coupled contactless conductivity detector ("C4D") near the outlet-well. The C4D consisted of two cuff electrodes around the capillary: a first cuff electrode that introduced an alternating voltage (300 Volts at 300 kHz), and a second cuff electrode 2 mm downstream, that sensed the alternating current in the solution (which is a very strong function of ionic concentration). The signal sensed by the C4D was fed to a custom amplification and signal conditioning circuit, digitized, and sent to a computer.
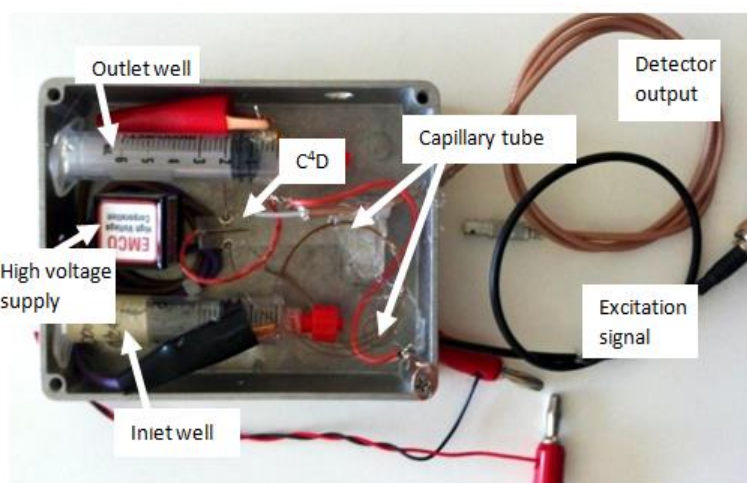

Figure 1. Capillary zone electrophoresis apparatus. 4"-long case houses coiled glass capillary tube, which is loaded from inlet well and dumps into outlet well under gradient established with high voltage. Miniature capacitive detector (C4D), mounted on capillary wall near outlet end, is activated by excitation signal from sine-wave generator, and output via cable to digitizer (Fig. 2).
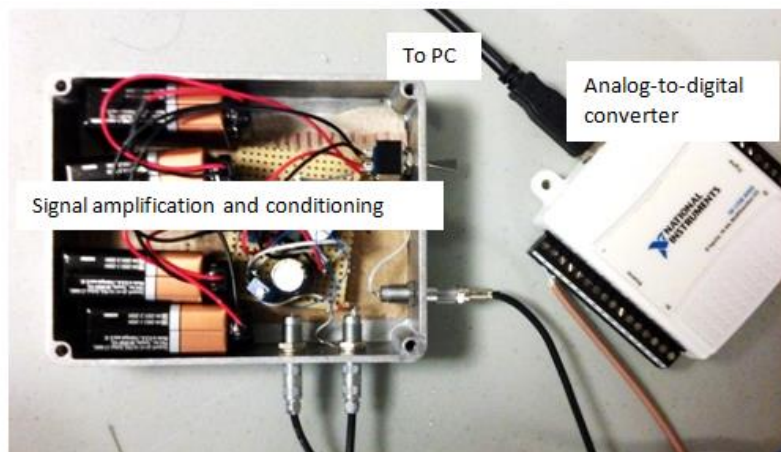
Figure 2. Readout electronics. Signal amplification and conditioning is housed in metal case to reduce influence of electrical noise. Analog-to-digital converter ships signal to PC.

To operate the apparatus, we pre-filled the wells and capillary with a background solution that did not contain Ge-68. We used dilute HCl (10-100 mM) as background solutions, at concentrations comparable to those used in commercial Ga-68 generators to elute the radionuclide. After pre-filling with dilute HCl, we introduced the sample to be tested for Ge-68 into the well connected to the positive terminal of the high-voltage power supply. When high voltage was applied to the wells, the ions in the sample moved through the capillary, towards the well connected to the negative high terminal. The capacitatively-coupled contactless conductivity detector (positioned along the capillary, close to the negative well), detected the presence of the ionic species as they passed by.

Calibrating the System. Although the end-purpose of the project was to detect radioactive Ge-68, we spent some time looking for methods of calibrating the system with non-radioactive forms of ionic germanium. This strategy allowed us to vary the concentration of germanium much more effectively than we would have been able to with a single generator, which helped us to better understand germanium chemistry. Due to the relative inertness of germanium, its ambiguous valence states (which are highly dependent on ambient pH), and its occasional tendency to form covalent as well as ionic bonds, there is scant modern literature about its chemistry. By the end of the study we found that we were educating representatives of generator companies as to the chemical states of the material. After extensive literature searching and experimentation of sources for Ge(IV), we selected HCl-dissolved GeO_2 as source of Ge^{+4} ions.

Novel Scientific Data for Germanium. Fig. 3 demonstrates the relationship between the conductivity measurement obtained with the system using the capacitive probe C4D and the Ge^{4+} concentration in dilute HCl. As one would expect, the curve converges to the value of conductivity at infinite dilution, and is consistent with similar curves for other ion species that have been published and are used in the water purification industry.
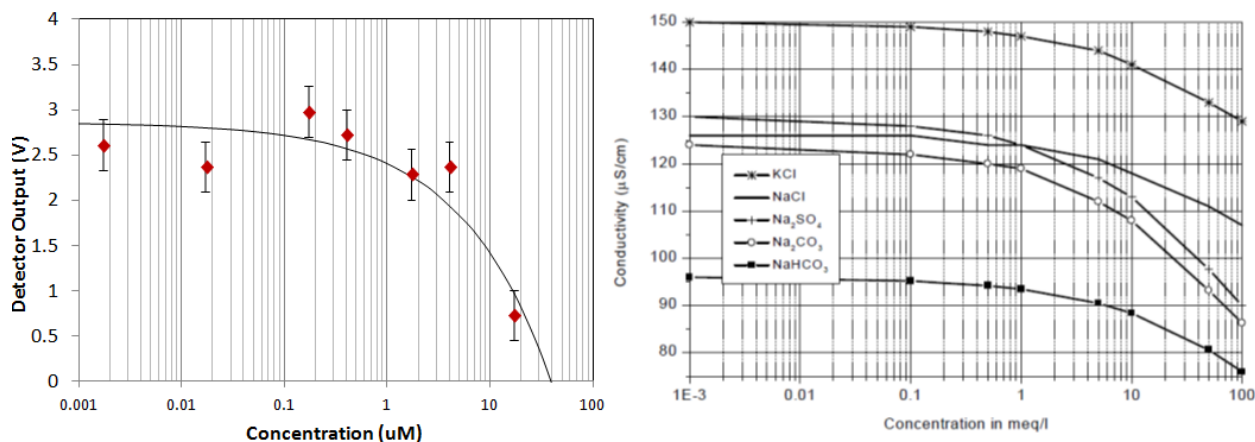
Figure 3. Conductivity measurements for Ge^{+4} solution. Left graph shows measured voltages (proportional to conductivity) for germanium. Right graph shows classic conductivity measurements for salt concentrations.

To our knowledge, the conductivity at infinite dilution for germanium had never been previously measured, and we have submitted this datum for scientific publication to the Journal of Nuclear Medicine. Upon testing with the standardized GeO$_2$-derived solutions, the miniature CZE system of Fig. 1 was able to detect the presence of Ge^{4+} ions in HCl solution at remarkably low (i.e., nM) concentrations with high repeatability.

An important parameter in electrophoretic separations is the mobility of the ions separated, because they guide the design of the apparatus (the mobility determines the ion arrival time at the end of the capillary, and ions must have different mobilities in order for them to be separated). To our knowledge, the mobilities of germanium and gallium ions were not known. We therefore had to measure them ourselves with our CZE system. Fig. 4 shows typical results on the arrival of Ge^{4+} and Ga^{3+} ions passed the capacitive probe, with the time delay reflecting the difference in ionic mobility. We measured the mobilities for Ga^{3+} and Ge^{4+} to be 4.5×10^{-4} and 6.22×10^{-4} cm^2/(V·s) respectively. This novel data has also been submitted for scientific publication, and will be valuable as we attempt to further improve the system in Phase II.
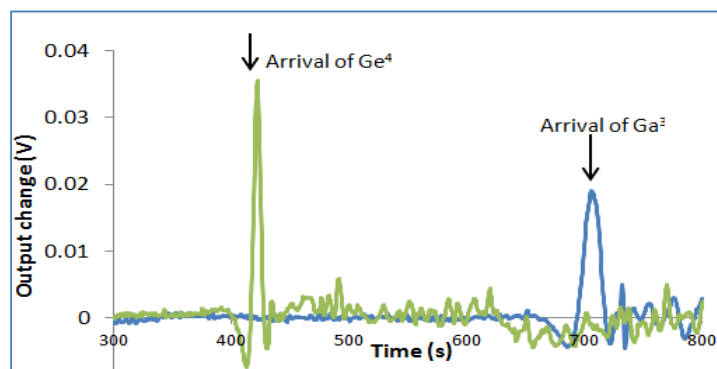

Figure 4. Mobility study. Higher charge of Ge^{+4} results in earlier arrival at C^{4}D sensor than Ga^{+3} ions.

Testing with Commercial Ga-68 Generator. Having calibrating the system with non-radioactive standard solutions of Ge^{+4}, we moved on to the "acid test" using a commercial Ga-68 generator obtained under our Radioactive Materials License (Model C2006-10, Cyclotron Co. Ltd., Obninsk, Russia).

We induced breakthrough of Ge-68 in the generator by using HCl of higher concentration (i.e., 1 M HCl) than that suggested by the manufacturer (100 mM HCl). Generators are well known to produce higher breakthrough when high HCl concentrations are used. In order to verify that breakthrough was present, we examined the eluent after ten half-lives. This testing was performed by consultant A&D Precision, since it is difficult to reliably obtain measurements at such low activity levels using standard dose calibrators. Using the apparatus of Fig. 1, we observed a significant increase in conductivity for the Ge-68 breakthrough case, as compared to a standard solution (Fig. 3).
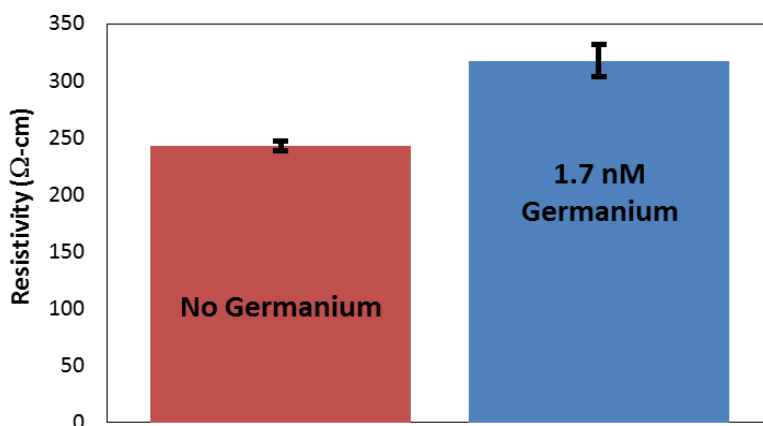

Figure 3. Radioactive Ge-68 measurement. System clearly detects presence of nanomolar concentrations of Ge-68.

4. Products developed/Conference Proceedings

Work was presented as a poster at the 2013 Meeting of the Society of Nuclear Medicine, in Vancouver, entitled: "Rapid Ge-68 breakthrough testing for 68Ge/68Ga portable generators", authored by: Mario Urdaneta, Pavel Stepanov, David Beylin, Roland Probst, Irving Weinberg.