Introduction

Lead contamination is ubiquitous in our environment and poses serious health risks for young children and pregnant women (1-2). Moreover, Blood Lead Levels (BLLs) as low as >10 ug/dL are linked to harmful consequences. The Kansas Department of Health and Environment (KDHE), an active participant in the Kansas Healthy Homes and Lead Hazard Prevention Program (KHHLHPP), screens and manages a database of lead-poisoned individuals (3).

Due to its lower detection capability, inductively coupled argon plasma mass spectrometry (ICP-MS) the preferred method for analyzing BLLs at 10 ug/dL. KDHE had previously automated a portion of the ICP-MS BLL analysis using an ESI SC-2 Autosampler. The SC-2 Autosampler accommodates up to four 96-well plates. Increased batch sizes and other changes requiring faster sample analysis time while maintaining low backgrounds necessitated introducing an automated sample preparation and assay plate setup system.

Blood spot extraction and BLL calibration standard setup for ICP-MS analysis are tedious procedures and sensitive to lead contamination. KDHE chose the JANUS workstation to automate these two manual processes. ICP-MS calibration standards are usually prepared manually once a week in 50 mL tubes and often must be replenished midweek when background lead levels begin to increase above accepted values. As the JANUS workstation quickly and easily prepares calibration standards in smaller 2 mL volumes, fresh ICP-MS standards can be made each day, reducing the need to repeat instrument calibrations.

This application note describes how a JANUS workstation was used by KDHE to prepare extracts from dried blood card punches and/or whole blood samples for trace metal analysis. Using an automated liquid handling instrument for processing these types of samples reduces the need for repeat instrument calibrations.

The JANUS® Automated Workstation is a flexible platform for preparing extracts from dried blood card punches and/or whole blood samples for trace metal analysis. Using an automated liquid handling instrument for processing these types of samples reduces the need for repeat instrument calibrations.

Application Workstations

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Materials and Methods

The overall process to prepare blood spot extracts for analysis at KDHE is shown in Figure 1.

Extraction and calibration standard protocols

The JANUS workstation was used to automatically prepare BLL sample extracts, transfer them into clean 96-well assay plates and prepare calibration standards. The same samples and standards were processed manually, in parallel. KDHE created separate WinPREP templates for the extraction (Figure 3) and calibration standard dilution (Figure 4) protocols.

Instrumentation

The JANUS workstation consisted of a Standard deck with Varispan™ 4-Tip arm (Figure 2). Blood spot punches were prepared using a PerkinElmer Autopunch™ first and later a BSD600-Duet™ puncher. BLL extracts were processed using an ESI SC-2 autosampler.

Figure 1. Automated blood lead analysis scheme. Reagents, standards and controls were prepared as per KDHE Test Method CM 202.01 (5). Punches (6 mm) from bar-code labeled blood-spotted cards were placed, either manually or automatically, using an Autopunch, in Costar 96-well diamond-bottom 2.2 mL deep well plates. User places the sample plate on an automated shaker on the JANUS deck and starts the protocol. Following assay setup, samples are analyzed using the ICP-MS. Positive and negative controls were included with each sample batch.

Figure 2. JANUS Standard platform with Varispan arm. Accessories included 500 uL syringes, VersaTip® Plus Option, VARIOMAG® Monoshake® and Teflon®-coated Waste Chute. Consumables included reagent troughs and 25 uL, 175 uL and 1000 uL conductive disposable tips.

Figure 3. Template for BLL punch extraction procedure. User choices include varying sample number and whether or not to make standards.

Figure 4. Template for standard dilution procedure. JANUS dilution protocol exactly duplicates manual CDC-validated KDHE scheme. It uses a 100 ppb stock to create six calibration Pb standards ranging from 1-40 ug/L, Calibration Check standard and blank control.
Sample tracking

WinPREP software tracks sample IDs across three different instrument platforms: BSD Duet Autopunch, JANUS workstation and an ICP-MS instrument (see also Figure 1). A custom WinPREP DLL is called at the start of the JANUS extraction protocol to open and convert a selected BSD600 punch data file into an output “BloodPb.csv” file (Figure 5). Script functions in subsequent WinPREP steps access output data to track sample identity and corresponding well map locations on the JANUS deck during assay plate setup.

Results

A typical automated standard curve using Pb standards prepared by the JANUS is shown in Figure 6.

JANUS studies included side-by-side comparisons of manual versus automated liquid handling setup, proficiency (samples obtained from the Wisconsin State Laboratory of Hygiene (WSLH) and other QC analysis. Typical assay results are shown in Figure 7 (A & B) and demonstrate that the JANUS workstation and manually processed samples gave similar results.

The JANUS Automated Workstation successfully automated the following steps in the BLL analysis processes:

(i) Extraction of blood card punches in deep well plates.

(ii) Addition of internal standards, required for downstream ICP-MS data evaluation, to each extracted sample. Reagent was aspirated using JANUS Accusense™ liquid level tip sensing to minimize carryover and improve precision.

(iii) Setup of an ICP-MS assay plate and a diluted standard and calibration control plate.

(iv) Sample ID tracking of bar-code labeled card punches throughout the assay in ICP-MS-compatible file format.

Figure 5. WinPREP User Program calls up a DLL function to generate output csv file. Output data file tracks the source plate name (A), well map location (D), Autopunch sample ID (E) and ICP-MS file format used by the ICP-MS (J).

Figure 6. Automated ICP-MS calibration curve. Calibration standards were prepared by JANUS from a 100 ppb stock Pb standard using same diluent and internal standard as for BLL samples. The main graph shows an ICP-MS standard curve for one lead isotope (Pb 208). Graph inset shows a composite plot for all three Pb isotopes (208, 207 & 206) present in the calibration standards as a function of observed versus expected Pb ug/dL levels.

Figure 7. A: JANUS automated (Result) compared with manually (Target) prepared BLL samples. B: WSLH proficiency analysis data.
The JANUS workstation successfully duplicated functions present in KDHE’s validated manual BLL analysis procedure. Increased throughputs of at least 4-fold were observed for single 96-well plate processing. Manual operation typically involves processing two batches of 60 tubes in an 8 h day (120 samples). The Workstation processed a 96-well sample plate in 60 min or less. The protocol is easily scalable. By adding a 4-plate shaker option, the workstation can process four plate batches per protocol. An integrated PlateStak™ microplate handler and storage device (5) offers even greater automated throughput capability.

Conclusions

The results show how the JANUS workstation is an efficient and flexible tool for automating trace metal analysis, in this case, BLL extraction and preparation for analysis. The Workstation offers other features ideal for environmental and health laboratories:

- Simple setup fully automates both extraction and preparation of ICP-MS calibration standard protocols in a variety of tube and plate (24-, 48-, 96-well) formats.
- The JANUS workstation increased throughput of at least 4-fold compared to manual setup.
- Flexible WinPREP software allows easy user programming, preserving current manual SOP parameters, thereby reducing time-consuming validation efforts.
- Validation studies showed that JANUS data is comparable to manual data.
- Disposable tips minimize metal leaching from metal tips under acidic conditions used for assay setup. Trace metal backgrounds were as low as manual processing. No metal contamination was found.

- Samples and calibration standards can be prepared in the same 96-well plate by combining both protocols into a single JANUS template.
- Automated operation minimizes user error and is as accurate as manual BLL assays.

The JANUS workstation can be integral to many trace metal analysis schemes by automating entire metal screening panels using dried spot, blood, serum, urine or samples.

References


