Suitability of SASS 2300 Sample Vials and SASS 3010 Sample Vials for use with Hand-Held Assays

1.0 Introduction

The SASS® 2300 air sampler incorporates a Vial Filling Option that, following extended collection of particulates from air, allows the subsequent transfer and storage of the concentrated sample fluid in a small vial for later analysis with hand-held assays (HHAs) or other post-collection assay methods. Since most of these assay techniques require that the sample be presented in a buffered fluid format, Research International developed a sample vial that contains a small amount of lyophilized buffer salts. The SASS (Smart Air Sampler System) samples and collects with distilled water. When the nominal volume (5 mLs) of this sample water is added to the vial, the correct buffer concentration is achieved after mild agitation.

The SASS® 3100 dry air sampler collects particulates onto a filter which is then extracted with a proprietary buffer and stored in a dropper vial by the SASS 3010 Extractor.

Both instruments thus provide a sample ready to apply directly to DoD HHAs or other lateral flow immunoassay tickets, such as the BioThreat Alert product from Tetracore.

This document describes tests that certify the adequacy of samples prepared by the SASS 2300 Vial Filling Option or the SASS 3010 Extractor for use with HHA tickets.

2.0 Dropper Vial Qualification

Sample vials for use with the SASS 2300 are 8 mLs in volume to coincide with the nominal 5 mL capacity range of the SASS 2300, instead of the 4 mL size used in the Hand Held Assay (HHA). Sample vials for use in the SASS 3010 are 15 mLs in volume and contain 8 mLs of buffer for better extraction of particulates from the filter. Since manufacturers of the HHAs indicate it is very important that the correct amount of sample be applied to the HHA (3-4 drops, 80-120 uL typical), it was necessary to qualify the 8 and 15 ml dropper vials selected for use. This was done as follows:

Three DoD HHA buffer vials were selected for use as standards. Three each, 8 ml vials and 15 mL vials were randomly selected from a supplier shipment. The 8 mL vials were filled with 5 mLs of phosphate buffered saline, pH 7.4 containing 0.05% Triton X-100. The 15 mL vials were filled with 8 mLs of SASS 3010 Extraction Buffer Kit (Part Number 7000-162-134-02). Simulating field operation, three drops from each vial were squeezed into a bottle on an analytical balance and the weight increase recorded. A fourth drop was dispensed and the weight increase recorded. The tests were repeated four times for each vial for a total of five trials.

2.1 Dropper Test Results

Table I summarizes the test results. The HHA vial transferred about 10 uL less than did the larger vials. However, given the HHA instructions (add 3-4 drops to the ticket), this difference is small. Whether using SASS 2300 8 mL vials or SASS 3010 15 mL vials, adding 3-4 drops of sample to the ticket will meet assay requirements.

Table I

Results of Equivalence Test on 4 ml and 8 ml Dropper Bottles

Dropper Bottle	Average volume in three drops (uL)	Average volume in four drops (uL)	Average drop volume (uL)
HHA vial #1:	72.4 ±8.2	97.4 ±8.1	24.2
HHA vial #2:	81.0 ±1.9	107.0 ±2.7	26.9
HHA vial #3:	78.8 ±4.7	104.2 ±4.2	26.2
HHA Averages	***77.4 ±6.4***	***102.9 ±6.6***	***25.8***
8 ml vial #1:	85.4 ±9.5	113.8 ±9.5	28.5
8 ml vial #2:	91.6 ±2.4	119.2 ±4.2	30.2
8 ml vial #3:	86.0 ±5.8	114.6 ±6.5	28.7
8 ml Averages	***87.7 ±6.7***	***115.9 ±7.0***	***29.1***
15 ml vial #1:	89.6 ±3.1	115.0 ±7.4	29.3
15 ml vial #2:	88.2 ±5.4	119.2 ±5.9	29.6
15 ml vial #3:	81.4 ±5.4	105.8 ±7.7	26.8
15 ml Averages	***86.4 ±5.8***	***113.3 ±8.7***	***28.6***

3.0 Lyophilized Buffer Assay Tests

Sample vials for use with the SASS 2300, contain lyophilized buffer that is re-hydrated with the DI water from the collector. This approach prevents any dilution of the collected sample prior to analysis. It was necessary to verify that buffer can be prepared, lyophilized and re-hydrated to provide pH 7.4 buffer compatible with the Hand Held Assay (HHA). HHA buffer solution was prepared at Research International using 1/10 the water called for to make a 10X concentrated solution. Triton X-100 was added to provide the necessary surfactant. No biocide was added the buffer was lyophilized in the sample vials obviating concerns about microbial growth during storage. Biocide may be added if samples will be collected and held for a period of time before testing.

Five vials were charged with 0.5 mLs of the 10X buffer, frozen in liquid nitrogen and lyophilized over a four-hour period. Each vial was placed in a SASS 2300 and charged with a nominal 5 mLs of DI water from the cyclone tube. Each vial was weighed to determine the amount of water added and the pH was measured.

Vial 1	4.1 mLs	pH 7.43
Vial 2	4.6 mLs	pH 7.46
Vial 3	5.0 mLs	pH 7.46
Vial 4	4.2 mLs	pH 7.44
Vial 5	4.7 mLs	pH 7.46

The pH for all vials was within tolerance for the HHA system.

3.1 Simulant Bioassay Tests

To complete qualification of the SASS buffer, it was necessary to show that an HHA could be performed with a bioagent-spiked DI water sample of the type that would be produced by the SASS. To achieve this, assays for *Bacillus globigii* (Bg) were performed with Bg HHA kits (lot number 308050, manufactured 8/03) and Research International's lyophilized buffer.

Standards of Bg spores (spores provided by Dugway Proving Ground) in DI water were prepared by serial dilution ranging from 1×10^7 to 1×10^3 spores/mL. Samples were prepared by adding 5 mLs of Bg standard to a vial of lyophilized buffer. Test results were as follows:

Test 1	1×10^7 spores/mL	HHA failed mechanically – no liquid transport
Test 2	1×10^7 spores mL	positive
Test 3	1×10^6 spores/mL	positive
Test 4	5×10^5 spores/mL	positive
Test 5	1×10^5 spores mL	positive (LOD)
Test 6	5×10^4 spores/mL	negative
Test 7	1×10^4 spores/mL	negative
Test 8	1×10^5 spores mL	positive (LOD)
Test 9	1×10^5 spores mL	positive (LOD)

The LOD is determined to be 1×10^5 spores/mL in these tests.

Control

0.1 mLs of Bg diluted into 4.9 mLs of HHA buffer from kit

Test 10	1×10^5 spores/mL	positive
Test 11	5×10^4 spores/mL	negative

3.2 Buffer Concentration Effects

SASS users may desire to use the sampler with a water volume that differs from the factory default 5 cc setting. To verify that any resulting changes in buffer concentration do not affect HHA bioassay test results, the tests were repeated with a range of sample fluid volumes.

A Bg standard of 1×10^5 spores/mL was prepared in DI water. Since this concentration is the measured LOD for the HHA in our hands, it is the most crucial concentration level at which such a test can be performed. Various volumes of this spiked sample material were added to vials of lyophilized buffer and tested with the Bg HHA kit.

Test 1	4.0 mLs	positive
Test 2	4.5 mLs	positive
Test 3	5.0 mLs	positive
Test 4	5.5 mLs	positive
Test 5	6.0 mLs	positive

Control samples containing no Bg were also tested at the buffer concentration limits and no false positives were observed. In addition, tests were performed at each buffer concentration with the standard 8-agent HHA kit, and no false positives were observed for any agent over the buffer range examined.

4.0 Conclusion

Tests were performed to certify that the SASS 2300 system with the Vial Filling Option and the SASS 3010 Extraction Buffer and Vial could be used as a platform for HHA ticket assays. These tests involved HHA sample volume delivery tests for the baseline dropper vial; limit-of-detection tests with Bg HHA's and vials pre-charged with lyophilized buffer salt; and limit-of-detection/false positive tests for vials intentionally under- or over-filled. Test results can be summarized as follows.

Both dropper bottles provide either 3 or 4 drop samples that are well within the 80-120 uL requirement for HHA samples. Limit-of-detection tests with Bg produced results that were indistinguishable from tests with non-lyophilized buffers. Over a 4 to 6 ml sample volume range, which represents expected SASS under- and over-fill limits, no effect of buffer concentration on LOD was observed with the Bg tickets. The various buffer concentrations also did not produce false positives with either the Bg HHA tickets or 8-agent HHA kits.