AUTOSTART BURETTE PARTICULATE CONTAMINATION TESTING

INTRODUCTION

The following validation test was performed to demonstrate the AutoStart Burette's compliance with the particulate contamination requirements of ISO 8536-4:2004.

Relevant excerpts from the standard may be found in Appendix 1.

The testing was performed by Mr Geoff Daly, Operations Manager, Analytica Ltd, on 25 October 2008.

APPARATUS

Figure 1: Apparatus

- An IV bag of distilled water (British Pharmacopoeia standard) - Baxter AHB0304 "Water for injection (BP)" 1000mL - Batch P85A3 - Expiry Aug 09.
- 10 new Autostart Burette devices. Batch 200809.
- An IV pole capable of about 2m height.
- Pall Solvac filter holder (capable of holding 47mm membrane filters) - LOT 072208.
- Pall HT450 Tuffryn membrane filter 47mm diameter, 0.45micron, Pall part number 66223, LOT T80708.
- 500mL measuring vessel.
- Vacuum tubing.
- Vacuum pump with gauge.

- Pall Analyslide petri dishes (Part Number 7231), LOT 080905G011.
- Tweezers
- Microscope with digital camera.
- Micrometer slides, for image calibration.
- Particle size analysis software (ImageJ)

METHOD

The method is based on that described in Annex A.1 of the standard.

A summary of the method is to run distilled water through 10 ASB units in sequence, which is then filtered through a membrane serving as the particle collection agent. This membrane is then studied for particle size. The specific steps are as follows:

Four ASB devices were connected in series underneath the fluid bag hanging from the IV pole (Figure 2).

Using the clamps and air vents accordingly, the devices were then filled with the distilled water, requiring a total of approximately 640mL of fluid (160mL x 4).

A fifth device was then attached to the bottom of the series and the fluid bag at the top removed. The fifth ASB was filled with water by allowing the fluid to fill down from the chain of units. When the top device emptied except for the anticipated few mL of residual volume, it was removed from the series and the next one attached to the pole (Figure 3).

The process continued in this fashion until the 10th device was added.

At the outlet of the 10th device the Solvac membrane filtration unit was fitted onto the end to filter the particles.

Underneath the filtration unit the measuring vessel was placed to collect 500mL of fluid. Refer Figure 4.

Fluid was allowed to flow through the 10th device until 500mL was collected downstream of the vacuum filter.

The filter membrane was then placed in an Analyslide petri dish for subsequent microscopy.

Fluid in excess of the required 500mL was discarded, and the devices used for other mechanical tests.

Figure 2: Initial test setup with fluid bag and IV pole.

Figure 3: Test setup without fluid bag.

Figure 4: Final arrangement with filter and collection vessel.

RESULTS

Calibration

Analysis

As set out in the Standard, the analysis involves measuring the particles according to size categories given in Table 1 below:

Table 1: Evaluation of contamination by particles

		Size category	
Particle parameters	**1**	**2**	**3**
Particle size in µm	25 to 50	51 to 100	over 100
Average number of particles in 10 infusion appliances	n_{a1}	n_{a2}	n_{a3}
Average number of particles in the blank control samples	n_{b1}	n_{b2}	n_{b3}

Evaluation coefficient	0,1	0,2	5

For each of the three size categories, the resultant average number of particles is multiplied by the corresponding evaluation coefficients and added together to obtain the overall particle count, N_a.

The filter is then assessed according to the "contamination index", N, as mentioned in the compliance statement, which is measured by subtracting N_b from N_a (N_b is the number of particles in the blank control sample), and $N = N_a - N_b \leq 90$.

Evaluation

For the evaluation, the membrane filter was placed on an Analyslide petri dish and the cover placed on the dish.

The membrane filter was then scanned left-right, then down by one height of the field of view, then right to left, then down one field of view, and so on.

When an object came within the field of view, an image was captured using the digital camera. Examples of distinct objects are shown in Appendix 2.

406 particles were found on the membrane filter.

The particle images were analysed using the ImageJ software package. The software package was calibrated using the micrometer slides shown below.

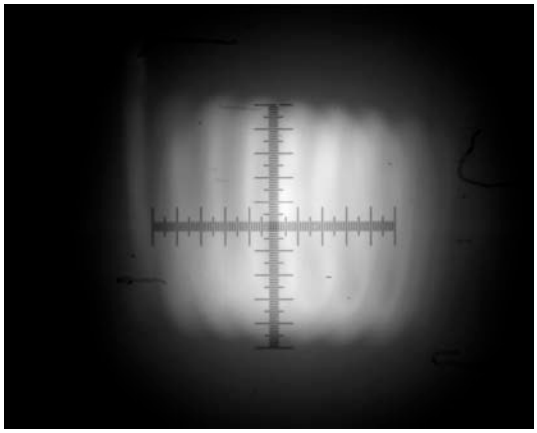

Figure 5: 1mm x 1mm crosshair calibration slide.

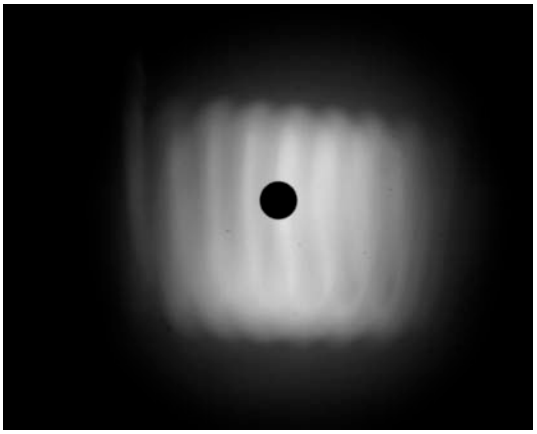

Figure 6: 0.15mm diameter calibration dot.

The crosshairs on the calibration slide shown in Figure 5 are 1mm x 1mm. The ImageJ software calibration was 586.67 pixels/mm. Performing an image analysis on the 0.15mm calibration dot shown in Figure 6, the software gave the dot's diameter as 0.1582. This overstates the size by 5.3% so the analysis is conservative.

The standard does not specify how to measure "particle size" so for this test the maximum Feret diameter was chosen. This is the most conservative approach.

The data is summarised in the size distribution of Table 2.

Table 2: Particle count result

Size Range	Count (10 devices)
0-25µm	75
25-50µm	125
50-100µm	107
>100µm	99

Note that although the number of particles of size 0-25µm was recorded, it is not required to obtain the contamination index.

Number of particles in the infusion appliances (test pieces):

$N_a = n_{a1} \times 0{,}1 + n_{a2} \times 0{,}2 + n_{a3} \times 5$

$N_a = 1/10 \times (125 \times 0.1 + 107 \times 0.2 + 99 \times 5)$

$N_a = 52.89$

This resulting number demonstrated that there is no need to analyse the blank control sample, as:

$N = N_a - N_b \leq 90,$

which means that even without the reduction offered by subtracting the blank sample, the test is a pass – the contamination index is well below the allowed 90.

CONCLUSION

This result demonstrates that the AutoStart Burette complies with the requirements of ISO 8536-4:2004(E), Section 6.1 Particulate Contamination.

APPENDIX 1 – ISO 8536-4:2004 EXCERPT

ISO 8536-4:2004 Infusion equipment for medical use — Part 4: Infusion sets for single use, gravity feed:

6.1 Particulate Contamination

The infusion set must be manufactured under conditions that minimise particulate contamination. All parts shall be smooth and clean at the fluid pathway surfaces. When tested as specified in A.1, the number of particles shall not exceed the contamination index."

A.1 Test for particulate contamination

A.1.1 Principle

The particles are rinsed from the inner fluid pathway surfaces of the infusion set, collected on a membrane filter and microscopically counted.

A.1.2 Reagents and materials

- A.1.2.1 Distilled, 1,2 µm filtered water.
- A.1.2.2 Non-powdered gloves.

A.1.3 Procedure

Flush through 10 ready-to-use infusion appliances under laminar flow conditions (clean-air work station class N5 according to ISO 14644-1) with 500 ml of distilled water previously filtered through a membrane of pore size 0,2 µm. Then draw this distilled water by vacuum filter having a pore size of 0,45 µm. Place the particles on the membrane screen filter under a microscope at 50 times magnification using diagonally incident illumination, and measure and count in accordance with the size categories given in the Table A.1.

A.1.4 Treatment of results

A.1.4.1 General

Single infusion sets, appropriate in total number (minimum of 10) are tested. The average count of particles per infusion set tested in each of the three size categories is the assay result.

A.1.4.2 Particle counts

The values obtained from a blank control sample shall be recorded in a test report and taken into account when calculating the contamination index. The blank control sample is the average number and size of particles obtained from each of 10 equivalent 500-ml water samples

classified in accordance with the three size categories set out in Table A.1 using the same test equipment but not passed through the appliances under test. The number of particles in the blank (Nb) shall not exceed the value of 9. Otherwise, the test apparatus shall be disassembled, re-cleaned, and the background test performed again. Values of the blank determination are to be noted in the test report. The filter unit, filter, and all other equipment shall be thoroughly cleaned before the test using distilled water passed through a membrane filter of pore size 0,2 µm.

Table A.1 — Evaluation of contamination by particles

		Size category	
Particle parameters	1	2	3
Particle size in µm	25 to 50	51 to 100	over 100
Average number of particles in 10 infusion appliances	na1	na2	na3
Average number of particles in the blank control samples	nb1	nb2	nb3
Evaluation coefficient	0,1	0,2	5

The contamination index is calculated as follows.

For each of the three size categories, multiply the average number of particles in 10 infusion appliances by the evaluation coefficients, and add the results to obtain the number of particles in the infusion appliances (test pieces), N_a. Then, for each of the size categories, multiply the average number of particles in the blank control samples by the evaluation coefficients and add the results to obtain the number of particles in the blank sample, N_b.

Substract N_b from N_a to obtain the contamination index.

Number of particles in the infusion appliances (test pieces):

$N_a = n_{a1} \times 0,1 + n_{a2} \times 0,2 + n_{a3} \times 5$

Number of particles in the blank sample:

$N_b = n_{b1} \times 0,1 + n_{b2} \times 0,2 + n_{b3} \times 5$

Contamination index:

$N = N_a - N_b = 90$

APPENDIX 2 – EXAMPLE PARTICLE IMAGES