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Research Article

Cascade Impactor (CI) Mensuration—An Assessment of the Accuracy and Precision of Commercially Available Optical Measurement Systems

Frank Chambers,[1] Aziz Ali,[1] Jolyon Mitchell,[2] Christopher Shelton,[3,5] and Steve Nichols[4]

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Abstract. Multi-stage cascade impactors (CIs) are the preferred measurement technique for characterizing the aerodynamic particle size distribution of an inhalable aerosol. Stage mensuration is the recommended pharmacopeial method for monitoring CI "fitness for purpose" within a GxP environment. The Impactor Sub-Team of the European Pharmaceutical Aerosol Group has undertaken an inter-laboratory study to assess both the precision and accuracy of a range of makes and models of instruments currently used for optical inspection of impactor stages. Measurement of two Andersen 8-stage 'non-viable' cascade impactor "reference" stages that were representative of jet sizes for this instrument type (stages 2 and 7) confirmed that all instruments evaluated were capable of reproducible jet measurement, with the overall capability being within the current pharmacopeial stage specifications for both stages. In the assessment of absolute accuracy, small, but consistent differences (ca. 0.6% of the certified value) observed between 'dots' and 'spots' of a calibrated chromium-plated reticule were observed, most likely the result of treatment of partially lit pixels along the circumference of this calibration standard. Measurements of three certified ring gauges, the smallest having a nominal diameter of 1.0 mm, were consistent with the observation where treatment of partially illuminated pixels at the periphery of the projected image can result in undersizing. However, the bias was less than 1% of the certified diameter. The optical inspection instruments evaluated are fully capable of confirming cascade impactor suitability in accordance with pharmacopeial practice.

KEY WORDS: cascade impactor; inhaler testing; optical inspection; performance verification; stage mensuration.

INTRODUCTION

The measured aerodynamic particle size distribution (APSD) of emitted aerosol from an oral-inhaled product (OIP) is a key performance parameter for assessing the ability to deliver a therapeutic dose of the medication to the lungs (1,2). Current pharmacopeial methods offer the option of several multi-stage cascade impactors (CIs) or the multi-stage liquid impinger (MSLI) that can measure the APSD of these aerosols with direct traceability to mass of active drug substance (3). Their principle of operation is the size fractionation of the incoming aerosol from the OIP based on differing particle inertia, a process that is described elsewhere and is well understood (3–5).

In the context of understanding sources of measurement variability associated with APSD testing, Nichols observed that, in the case of the Next Generation Pharmaceutical Impactor (NGI), the contribution to overall error in the inhaler aerosol APSD measurement process associated with assuring aerodynamic performance by calibration or stage mensuration was only ±1%, as compared with ±3% for internal losses, ±5% for environmental factors, ±5% for the setting of flow rate, and as much as ±20% for the intrinsic variability associated with drug product dose uniformity (6). The NGI is likely to be representative of other multi-stage CIs in these respects, given their common origins. Nevertheless, ways to determine aerodynamic performance that are fully traceable ultimately to the SI length standard are required in order to operate such equipment in a GxP environment (7).

CI/MSLI (referred to as CI from now onwards) performance was traditionally assessed by the laborious process of individual stage calibration using many sizes of spherical, monodisperse particles (8,9). More recently, the measurement of critical stage dimensions (stage mensuration) has been adopted as a more efficient and practical procedure (10). Primarily, it is individual jet (nozzle) diameters that determine the aerodynamic size-selectivity of a particular stage. Diameters may be measured directly or calculated from the cross-sectional area of the jet. Once stage mensuration has taken place, measured jet diameters are compared to the design specifications for the CI under evaluation. This procedure is now the norm as the means of assuring CI aerodynamic performance routinely (11). Mensuration is also cited in the current European and US Pharmacopeias as the primary method for monitoring the "fitness for purpose" of CIs used within a GxP environment (12,13).

Roberts and Romay (14) recently showed that the aerodynamic performance of a multi-nozzle stage is described by a

[1] AstraZeneca Charnwood, Loughborough, UK.
[2] Trudell Medical International, London, Canada.
[3] PPD Inc., 8551 Research Way, Suite 90, Middleton, Wisconsin 53562-4664, USA.
[4] OINDP Consultant, Cheshire, UK.
[5] To whom correspondence should be addressed. (e-mail: christopher.shelton@ppdi.com)

single size-related metric termed 'effective diameter', a parameter calculated directly from stage mensuration data. Provided that the other more secondary aspects of impactor geometry are maintained (15), it is now possible to demonstrate traceability linking CI aerodynamic performance to the stage mensuration process through the use of certified reference standards that are commonly used to calibrate the optical image analyzers (inspection systems) used for mensuration. These standards are themselves ultimately traceable to the international length standard. Current compendial acceptance criteria for CI stages has thus far focused on the achievable tolerances in impactor manufacture, while conceding that alternative limits can be justified on a case-by-case basis (12,13).

Stage mensuration can also be performed using calibrated pin gauges for the largest jets, and historically such gauges were used as the primary means to measure jet diameter. Although pin gauges are easy to use and, as primary standards, are calibrated to a high degree of accuracy, there are limitations:

1. They are invasive
2. Accuracy of gauge-determined jet diameter is affected by the circularity of the jet
3. Manual use of gauges is tedious for stages containing hundreds of jets

More recently, optical systems, having software-driven image analysis capability, have become the preferred measurement instrument, since this equipment addresses most of the limitations of pin gauges, providing fast and accurate results from the many hundreds of repetitive measurements that are needed to complete the process for a typical CI (10), and in some cases offering the capability of quantifying jet circularity. However, the algorithms associated with the analysis software are proprietary, and therefore not well understood by the user community. This lack of transparency in a critical component of the measurement process may account for the fact that Shelton found observable differences in reported jet diameters for recently manufactured non-viable Andersen 8-stage CIs (ACIs) between three different measurement systems to assess the same stages (16). In the worst case associated with the assessments of the smallest nozzles (0.254 mm nominal diameter), extreme values of these differences approached 5% of nominal diameter. A more recent survey of members of the Impactor Sub-Team within the European Pharmaceutical Aerosol Group revealed that at least four different optical inspection systems are currently in use (17). It is, therefore, postulated that part of the reported variability in stage mensuration data may arise mostly from differences between optical inspection systems. To support this hypothesis, the Impactor Sub-Team undertook a round-robin study aimed at quantifying separately the measurement precision and accuracy of a variety of inspection instruments in use, with the objective of confirming the fitness of purpose of these systems for stage mensuration. This report describes the outcome of this investigation.

MATERIALS AND METHODS

Assessment of Measurement Precision

Stages 2 (400 jets, nominal diameter = 0.914 mm) and 7 (201 jets, nominal diameter = 0.254 mm) of a stainless steel ACI (Westech Instrument Services Ltd., Upper Stondon, Bedfordshire, UK) were chosen as reference articles, having representative large and small jet sizes, respectively (5). Each stage was examined independently in a round-robin exercise at nine different locations, some of which made their measurements

Table I. Overview of Optical Inspection System Characteristics

System Name	Parameter Measured	Manufacturer's Reported Diameter Reproducibility	Calibration Standard	Automation	Site Number	Study
Andersen Visual Inspection System (AVIS) (Specac, Ltd., Orpington, Kent, UK)	Area	0.005 mm	Gauge	☐Image Focus ☒Edge Detection ☒Image Analysis	1	☒Precision ☒Accuracy
			Gauge		2	☒Precision ☐Accuracy
			Gauge		3	☒Precision ☐Accuracy
			Gauge		4	☒Precision ☐Accuracy
Mondo Mondo Metrology Ltd. (no longer available) Site 5 = Model Optima 725 Site 6 = Model Optima 3	Diameter [a]	NA	Gauge	☐Image Focus ☐Edge Detection ☒Image Analysis	5	☒Precision ☐Accuracy
			Gauge		6	☒Precision ☐Accuracy
Mitutoyo QV404 (Mitutoyo UK Ltd., Andover, Hampshire, UK)	Circumference and Area	0.001 mm	Glass reticule	☒Image Focus ☒Edge Detection ☒Image Analysis	6	☒Precision ☐Accuracy
			Glass reticule		7	☒Precision ☒Accuracy
			Glass reticule		8	☐Precision ☒Accuracy
RAM Optical Datastar 100 (RAM Optical Instrumentation Inc., Rochester, NY, USA)	Circumference	0.001-0.008 mm, depending on stage	Glass reticule	☐Image Focus ☒Edge Detection ☒Image Analysis	8	☒Precision ☒Accuracy
RAM Omis II non-contact vision system (RAM Optical Instrumentation Inc., Rochester, NY, USA)	Circumference	0.001 mm	Glass reticule and gauge	☐Image Focus ☒Edge Detection ☒Image Analysis	9	☒Precision ☒Accuracy
OGP Smartscope Flash 500 (Optical Gaging Products, Inc., Rochester, NY, USA)	Circumference	0.001 mm	Glass reticule and gauge	☒Image Focus ☒Edge Detection ☒Image Analysis	9	☐Precision ☒Accuracy

[a] fully manual measurement

Fig. 1. Photo showing glass reticule slide (*left*) and calibrated ring gauges (*right*) in the specially designed holders for analysis. *Dark regions* in the glass slide are the transparent portions where no chromium deposits exist. Used with permission from (7)

with a different make or model of an inspection system as depicted in Table I. On receipt, each participant initially cleaned each stage using the procedure recommended by the supplier (Westech Instrument Services Ltd). The inspection system was initially calibrated using the procedure in use at that location, before triplicate measurements of the stages were made in the order: stage 2, stage 7, stage 2, stage 7, stage 2, stage 7. The following parameters were reported:

- Number of jets measured per stage
- Jet diameter
- Calculated jet area from diameter
- Measured jet area
- Calculated jet diameter from area

The measures of jet diameter and/or area, depending on equipment type, together with a brief description of their site-specific system calibration procedure, were forwarded by each participant to the study co-ordinator. This information included verification that length-traceable standards had been used to calibrate the equipment. The mean, median, range, % RSD, and effective diameter for each stage were calculated as descriptive statistics and the data sets containing the individual measures were also assessed for components of variability (Appendix) by two-way ANOVA using SAS v. 9.1 (SAS Institute Inc, Cary, North Carolina, USA).

Assessment of Measurement Accuracy

The accuracy of each inspection system was separately evaluated to understand sources of possible systematic differences observed by Shelton (16). It was postulated that differences in the treatment of partially lit pixels along the jet circumference may have introduced bias in reported diameters. In addition, the physical properties of the nozzles themselves, such as jet depth effects and machining imperfections revealed in the projected images for analysis, may also have been interpreted differently by each type of system.

Six individual instruments were evaluated among four of the nine participating sites (Table I), with analysis of length-traceable standards conducted by each participant according to their internal standard operating procedure. A certified glass reticule with traceability to the international standard meter (National Physical Laboratory, Middlesex, UK) was used as one measurement reference standard that was circulated to each of the four participants. The reticule comprised nine chromium-on-glass ‘dots’ and nine glass-on-chromium circular ‘spots’ arranged

Table II. Descriptive Statistics for Inter-instrument Comparisons from Projected Jet Diameters: AVIS Systems

ACI stage 2 AVIS projected diameter (mm)							ACI stage 7 AVIS projected diameter (mm)						
Site	Replication	Number	Mean	SE mean	SD	RSD (%)	Site	Replication	Number	Mean	SE Mean	SD	RSD (%)
1	1	400	0.9020	0.0002	0.0031	0.34	1	1	201	0.2600	0.0002	0.0033	1.27
	2	400	0.9022	0.0002	0.0030	0.34		2	201	0.2599	0.0002	0.0033	1.27
	3	400	0.9021	0.0002	0.0031	0.34		3	201	0.2601	0.0002	0.0032	1.24
2	1	400	0.9030	0.0001	0.0023	0.26	2	1	201	0.2515	0.0002	0.0028	1.11
	2	400	0.9024	0.0001	0.0023	0.25		2	201	0.2519	0.0002	0.0028	1.11
	3	400	0.9042	0.0001	0.0022	0.24		3	201	0.2516	0.0002	0.0027	1.06
3	1	400	0.9175	0.0001	0.0026	0.29	3	1	201	0.2591	0.0002	0.0035	1.35
	2	400	0.9175	0.0001	0.0026	0.28		2	201	0.2591	0.0002	0.0035	1.34
	3	400	0.9175	0.0001	0.0026	0.28		3	201	0.2591	0.0002	0.0035	1.36
4	1	400	0.9200	0.0002	0.0033	0.35	4	1	201	0.2573	0.0002	0.0027	1.05
	2	400	0.9160	0.0002	0.0030	0.33		2	201	0.2574	0.0002	0.0028	1.09
	3	400	0.9171	0.0002	0.0031	0.34		3	201	0.2565	0.0002	0.0028	1.10

Table III. Descriptive Statistics for Inter-instrument Comparisons from Measured Jet Diameters: Non-AVIS Systems

Site	Replication	Number	Mean	SE mean	SD	RSD (%)	Site	Replication	Number	Mean	SE mean	SD	RSD (%)
ACI stage 2 Mitutoyo diameter (mm)							ACI stage 7 Mitutoyo diameter (mm)						
6	1	400	0.9101	0.0002	0.0032	0.36	6	1	201	0.2555	0.0002	0.0030	1.18
	2	400	0.9101	0.0002	0.0032	0.35		2	201	0.2556	0.0002	0.0030	1.16
	3	400	0.9103	0.0002	0.0032	0.35		3	201	0.2555	0.0002	0.0030	1.17
7	1	400	0.9092	0.0001	0.0030	0.33	7	1	201	0.2549	0.0002	0.0032	1.24
	2	400	0.9091	0.0001	0.0030	0.33		2	201	0.2548	0.0002	0.0032	1.26
	3	400	0.9091	0.0001	0.0030	0.33		3	201	0.2549	0.0002	0.0032	1.26
ACI stage 2 Mondo systems diameter (mm)							ACI stage 7 Mondo systems diameter (mm)						
5 (Optima 725)	1	13	0.9078	0.0007	0.0026	0.29	5	1	8	0.2569	0.0015	0.0042	1.64
	2	13	0.9118	0.0009	0.0032	0.35		2	8	0.2543	0.0010	0.0029	1.13
	3	13	0.9141	0.0017	0.0060	0.66		3	8	0.2551	0.0011	0.0032	1.26
6 (Optima 3)	1	13	0.9210	0.0003	0.0067	0.73	6	1	8	0.2591	0.0012	0.0034	1.30
	2	13	0.9219	0.0003	0.0063	0.69		2	8	0.2601	0.0016	0.0045	1.74
	3	13	0.9213	0.0002	0.0049	0.53		3	8	0.2621	0.0017	0.0048	1.82
ACI stage 2 RAM systems diameter (mm)							ACI stage 7 RAM systems diameter (mm)						
8 (Datastar 100)	1	400	0.9177	0.0002	0.0032	0.35	8 (Datastar 100)	1	201	0.2595	0.0002	0.0031	1.20
	2	400	0.9171	0.0002	0.0030	0.33		2	201	0.2597	0.0002	0.0032	1.23
	3	400	0.9170	0.0001	0.0030	0.33		3	201	0.2592	0.0002	0.0031	1.19
9 (Omis II)	1	400	0.9226	0.0002	0.0033	0.36	9 (Omis II)	1	201	0.2580	0.0002	0.0026	1.02
	2	400	0.9229	0.0002	0.0032	0.35		2	201	0.2581	0.0002	0.0026	1.03
	3	400	0.9228	0.0002	0.0033	0.36		3	201	0.2580	0.0002	0.0027	1.04

in a parallel pattern, ranging in diameter from 0.254 to 5.5 mm (Fig. 1). This configuration allowed absolute assessment of instrument measurement accuracy based on a two-dimensional image profile. A specially designed holder, with exterior dimensions similar to the 8-stage ACI, was used to present the reticule consistently to each system.

Certified ring gauges having nominal diameters of 1.0, 2.5, and 4.5 mm (Alpha Gauging Ltd, UK) were also circulated as a second reference standard to evaluate the effect of three-dimensional profiles on accuracy of measured image (Fig. 1). The three ring gauges were placed in a separate designed ACI stage holder for consistent presentation to each system.

Ten replicate measurements of each dot/spot/aperture were obtained on three different days, resulting in 30 data points for each element. The accuracy associated with mean reported diameter was calculated for each calibrated dot/spot and ring gauge as a percent of the reported value from the associated certificate of analysis.

RESULTS

Measurement Precision

Descriptive statistics for measured jet diameter are reported in Table II (AVIS systems) and Table III (other

Table IV. Descriptive Statistics for Inter-instrument Comparisons from Jet Area Measurements

Site	Replicate	Number	Mean	SE mean	SD	RSD (%)	Site	Replicate	Number	Mean	SE mean	SD	RSD (%)
Stage 2 AVIS area (mm^2)							Stage 7 AVIS area (mm^2)						
1	1	400	0.6390	0.0002	0.0044	0.68	1	1	201	0.0531	0.0001	0.0013	2.53
	2	400	0.6392	0.0002	0.0043	0.68		2	201	0.0531	0.0001	0.0013	2.54
	3	400	0.6392	0.0002	0.0044	0.68		3	201	0.0531	0.0001	0.0013	2.47
2	1	400	0.6404	0.0002	0.0033	0.52	2	1	201	0.0497	0.0001	0.0011	2.21
	2	400	0.6396	0.0002	0.0032	0.50		2	201	0.0498	0.0001	0.0011	2.19
	3	400	0.6421	0.0002	0.0031	0.48		3	201	0.0497	0.0001	0.0011	2.13
3	1	400	0.6611	0.0002	0.0038	0.57	3	1	201	0.0527	0.0001	0.0014	2.70
	2	400	0.6611	0.0002	0.0038	0.57		2	201	0.0527	0.0001	0.0014	2.67
	3	400	0.6611	0.0002	0.0038	0.57		3	201	0.0527	0.0001	0.0014	2.71
4	1	400	0.6648	0.0002	0.0047	0.71	4	1	201	0.0520	0.0001	0.0011	2.11
	2	400	0.6590	0.0002	0.0044	0.66		2	201	0.0521	0.0001	0.0011	2.18
	3	400	0.6606	0.0002	0.0045	0.67		3	201	0.0517	0.0001	0.0011	2.20
Stage 2 Mitutoyo area (mm^2)							Stage 7 Mitutoyo area (mm^2)						
6	1	400	0.6461	0.0002	0.0043	0.67	6	1	201	0.0497	0.0001	0.0010	1.98
	2	400	0.6462	0.0002	0.0044	0.69		2	201	0.0497	0.0001	0.0010	2.00
	3	400	0.6465	0.0002	0.0043	0.67		3	201	0.0497	0.0001	0.0010	2.03
7	1	400	0.6442	0.0002	0.0040	0.62	7	1	201	0.0490	0.0001	0.0011	2.28
	2	400	0.6442	0.0002	0.0040	0.62		2	201	0.0490	0.0001	0.0011	2.28
	3	400	0.6442	0.0002	0.0040	0.62		3	201	0.0490	0.0001	0.0011	2.30

systems). Equivalent data for measured jet area are contained in Table IV for Mitutoyo and AVIS systems. Both methods for data reporting were needed because measurements made by the Mitutoyo systems could be provided either based on direct diameter measurement or computed from equivalent measurements of jet area. Furthermore, jet projected area was the primary measured parameter from the AVIS systems, and the reported jet diameters were therefore based on computed data, assuming that the image from each jet was perfectly circular in profile. The count-weighted mean stage jet diameter determined by each mensuration system (shown together with the corresponding values of count-weighted median diameter and effective diameter (14)), for stages 2 (Fig. 2a) and 7 (Fig. 2b) was within the compendial tolerance limits of ±0.0127 mm (either stage). However, the spread in these data was slightly wider with the Mondo equipment, possibly because only randomly located samples representing ca. 4% of the total number of jets/stage were assessed by these more operator-dependent, manually operated systems.

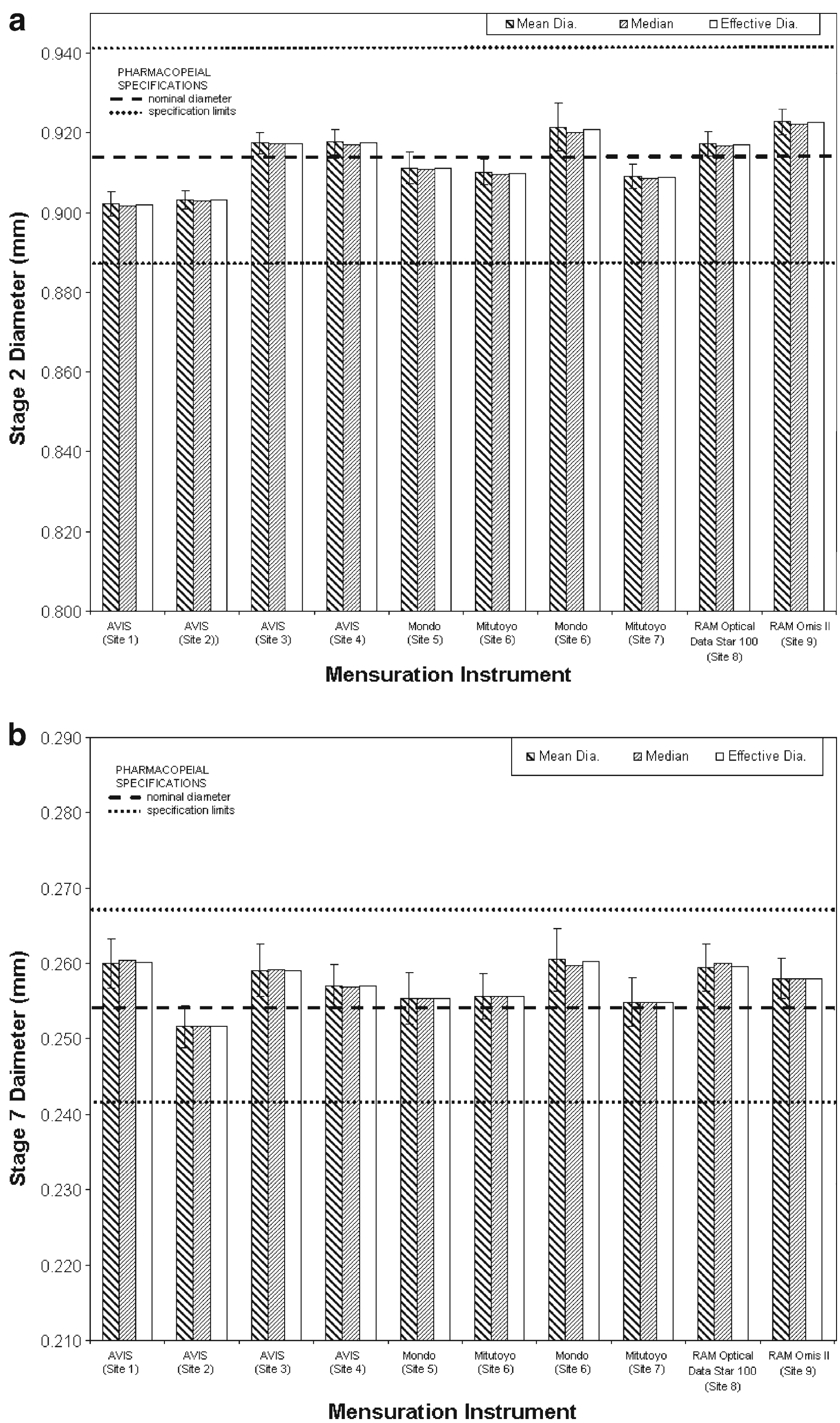

Fig. 2. Measured jet diameters of reference CI test stages (*error bars* represent 1 SD) for **a** stage 2 and **b** stage 7

Table V. Extreme Inter-site Values for Measured Jet Diameters

Stage 2: mean diameter (mm)			
Site number	Mean	Lower bound of 95% confidence interval	Upper bound of 95% confidence interval
9	0.9228	0.9226	0.9230
1	0.9021	0.9019	0.9023
Stage 2: extreme difference (mm)			
Difference	Difference	Lower bound of 95% confidence interval	Upper bound of 95% confidence interval
Site 9–Site 1	0.0207	0.0204	0.0209
Difference (% of nominal jet diameter)	2.26	2.24	2.29
Stage 7: mean diameter (mm)			
Site number	Mean	Lower bound of 95% confidence interval	Upper bound of 95% confidence interval
6 (Mondo)	0.2605	0.2587	0.2623
2	0.2517	0.2514	0.2519
Stage 7: extreme difference (mm)			
Difference	Difference	Lower bound of 95% confidence interval	Upper bound of 95% confidence interval
Site 6–Site 2	0.0088	0.0077	0.0100
Difference (% of nominal jet diameter)	3.47	3.01	3.92

Used with permission from (7)

There was no evidence of a consistent pattern of differences that could be linked to measurement system type (Tables II, III, and IV). The largest range in reported mean diameter for stage 2, representing 2.26% of nominal for this stage (0.914 mm), was obtained between participants 1 and 9 (Table V) using an AVIS and the RAM Omis systems, respectively. Similarly, the largest range in mean jet diameter values for stage 7, obtained between participants 2 and 6 using an AVIS and Mondo system, respectively, was 3.47% of nominal (0.254 mm). The magnitude of the 95% confidence interval associated with each mean jet diameter, taken as a measure of extreme variability in these measurements, represented <0.05% and <1.4% of the mean diameter for stages 2 and 7, respectively. Taking the smallest interval that enclosed both 95% confidence intervals from the individual site measurements to represent the overall divergence in measured jet diameter for each stage, the maximum difference occurred with stage 7, being equivalent to 3.92% of nominal.

Direct Jet Diameter Measurements

Further data analysis was possible for both AVIS, Mitutoyo, and Mondo systems, where an instrument of the same type was used at more than one laboratory (Appendix). Intra-instrument precision associated with the stage 2 AVIS measurements was estimated comparing RSD values for the replicates associated with a given instrument. This measure of precision was similar for each instrument, being clustered within narrow ranges typically within 0.02% of values in the overall range 0.24% to 0.35% (Table II). However, the absolute magnitude of the groups of mean jet diameters measured by the same instrument varied between participants. These differences (Fig. 3a) are indicative of significant inter-instrument variability, and further analysis (Table VI) confirmed that 89.1% of this variability originated from inter-site causes. While it is noted that the mean of the distribution of jet diameters in Fig. 3a is not centered within the range, this asymmetry is an artifact of the machining process for this stage, not the measurement process, as evidenced by the apparent symmetry in the jet distribution for the stage 7 and similarity of distributions between instruments.

The stage 7 AVIS-measured data also indicated that the precision associated with each inspection system was comparable for the three replicates (Table II), with the RSD for the measurements in the narrow range from 1.05% to 1.36%, approximately four times larger than equivalent RSD values for the larger stage 2 nozzles. This observation shows that the standard deviation of measurements is independent of jet diameter. There were, again, small differences detectable both in the absolute magnitude and spread of the diameter measurements between participating test sites (Fig. 3b), with

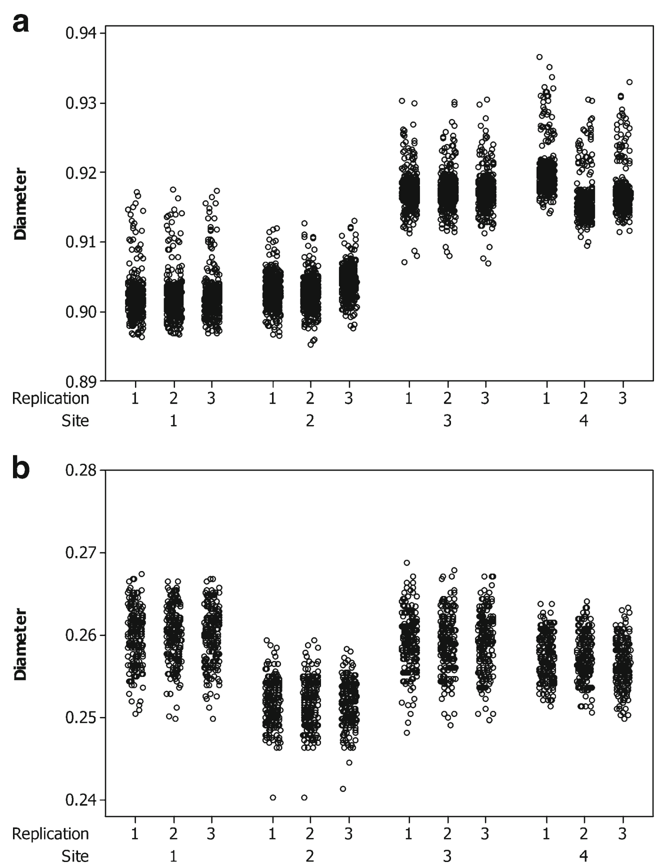

Fig. 3. Comparison of AVIS-projected jet diameters for **a** stage 2 and **b** stage 7

Table VI. Variability Components from Jet Diameter Data

Stage 2			Stage 7		
Variability source	Variability component	% of total	Variability source	Variability component	% of total
AVIS					
Inter-site	7.4×10^{-5}	89.1	Inter-site	1.3×10^{-5}	58.4
Replication	1.3×10^{-6}	1.5	Replication	2.7×10^{-8}	0.1
Residual component	7.8×10^{-6}	9.3	Residual component	9.6×10^{-6}	41.4
Total	8.33×10^{-5}	99.9	Total	2.3×10^{-5}	99.9
Mitutoyo					
Inter-site	5.0×10^{-7}	5.0	Inter-site	2.3×10^{-7}	2.4
Replication	UN	0.0	Replication	UN	0.0
Residual component	9.6×10^{-6}	95.0	Residual component	9.6×10^{-6}	97.6
Total	1.00×10^{-5}	100.0	Total	9.79×10^{-6}	100.0
Mondo					
Inter-site	5.0×10^{-5}	62.4	Inter-site	1.2×10^{-5}	44.0
Replication	3.0×10^{-6}	3.8	Replication	1.7×10^{-7}	0.6
Residual component	2.7×10^{-5}	33.8	Residual component	1.5×10^{-5}	55.4
Total	7.99×10^{-5}	100.0	Total	2.73×10^{-5}	100.0

UN = undetectable

58.4% of this variability originating from site-to-site causes (Table VI). It should be noted that the residual component comprising 41.4% of total variability, was higher than that associated for stage 2 (9.3%), and within expectation for a stage with smaller jets.

Similar analysis was undertaken for the data from the two participants using the Mitutoyo QV404 optical inspection equipment (Fig. 4a and b). While the overall variability of measurements from both locations was similar to that observed with the AVIS group, with the total RSD varying from 0.33% to 0.36%, the means and spreads of measurements between the two sites were comparable. Further analysis showed that only 5.0% of the total variability originated from inter-site differences (Table VI). A similar

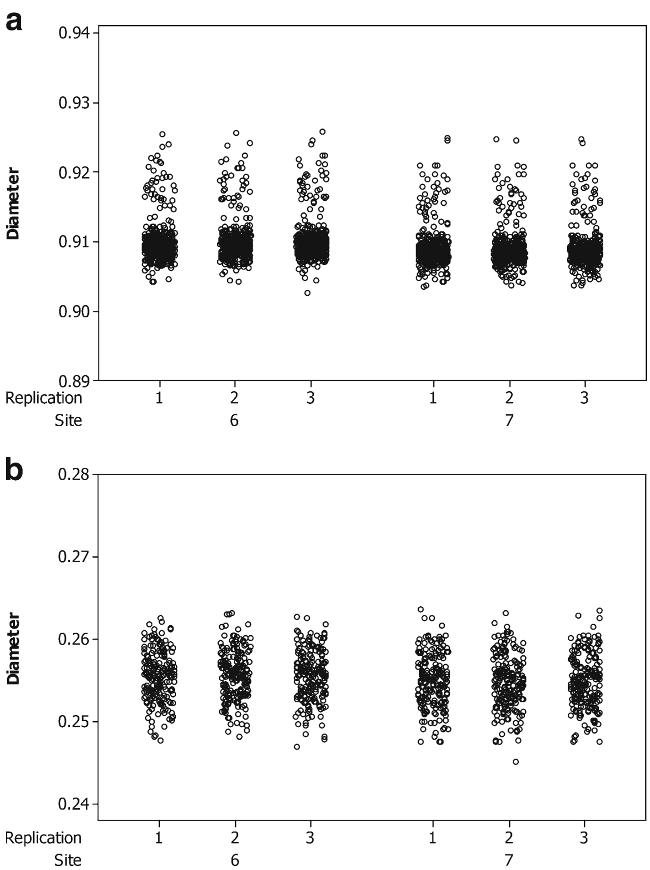

Fig. 4. Comparison of Mitutoyo QV404-measured jet diameters for **a** stage 2 and **b** stage 7

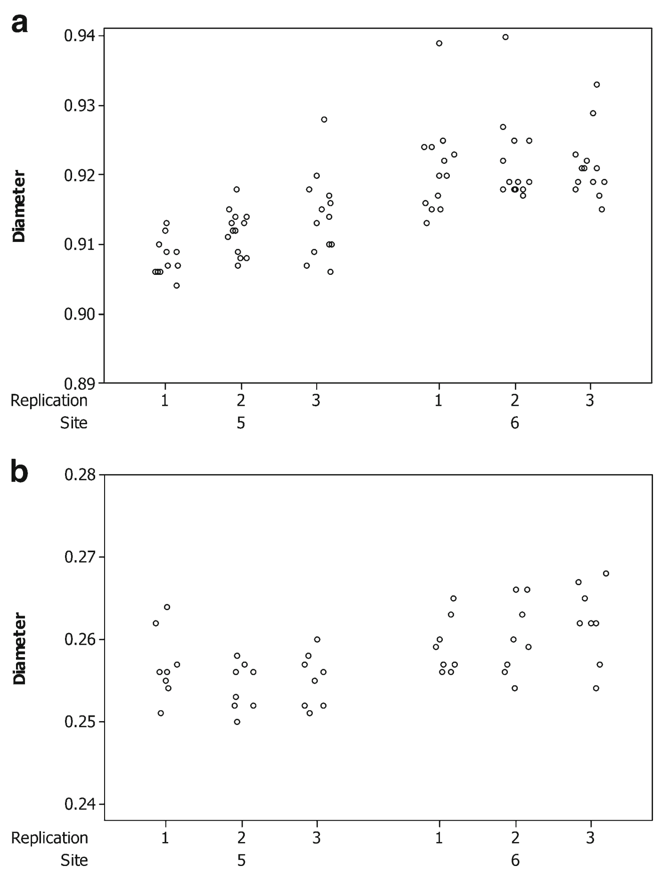

Fig. 5. Comparison of MONDO-measured jet diameters for **a** stage 2 and **b** stage 7

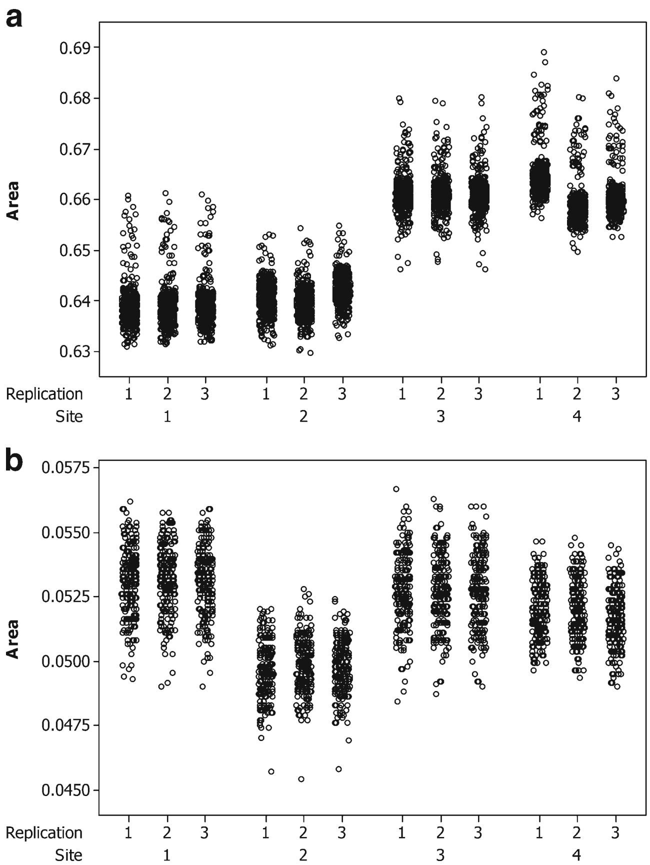

Fig. 6. Comparison of AVIS-measured jet areas for **a** stage 2 and **b** stage 7

pattern in the variability components occurred with the measurements of stage 7 by the Mitutoyo equipment, with total RSD values varying from 1.16% to 1.26% (Table III). Remarkably, the contribution to variability from replication with these instruments was too small to be detected in the analysis of data from either reference stage.

The total RSD from the Mondo-based measurements was 0.81% for stage 2, again indicating low overall variability (Table III). However, the intra-instrument precision for stage 2 was slightly more variable (Fig. 5a). ANOVA revealed that 62.4% of the overall variability originated inter-site with most of the remainder (33.8%) associated with the residual component (Table VI). Again, jet-to-jet differences (3.8%) were by far the smallest contributor to overall variability. It should be noted, however, that the measures for stage 7 with this manually operated equipment (Fig. 5b) were based on only eight determinations. Given the comparatively small sample size, estimates of the overall measurement variability had greater uncertainty associated with them, most likely accounting for the larger overall RSD value that ranged from 1.13% to 1.82% (Table III). Further analysis (Table VI) revealed that 44.0% of the total variability originated from site-to-site causes, with only 0.61% arising from replication.

Although inter-site analysis was not possible for the jet diameter measurements made by the two RAM inspection systems (Datastar 100 and Omis II operated at different locations), the overall variability associated with each analyzer for stage 2 (0.33% to 0.35% RSD for the Datastar 100; 0.35% to 0.36% RSD for the Omis) was comparable with the overall variability for the other three systems already considered (Table III). Similarly, overall variability ranges for stage 7 (1.20% to 1.23% for the Datastar; 1.02% to 1.04% for the Omis II) were at least as precise as equivalent data obtained from the other equipment.

Jet Diameters Computed from Image Area Measurements

Jet area measurements for stage 2 from the AVIS and Mitutoyo QV404 systems were analyzed by the same techniques used to assess the equivalent jet diameter data (Table IV). It is noted here that the AVIS instruments, due to design, only measure jet area, from which a projected diameter is calculated. While the jet diameter is the reported parameter, and thus relevant in the previous analysis, the jet area is the dimension actually measured. Measurement variability based on RSD values ranged from 0.48% to 0.71% (AVIS), and as expected, there were observable differences in both the absolute magnitude and spread of these area-based measurements between sites (Fig. 6a). Further analysis showed that 89.1% of the observed variation was attributable to site-to-site origins (Table VII). Similar behavior was observed with stage 7 (Fig. 6b), but the range in RSD varied from 2.13% to 2.71%, of which 59.0% could be attributed to site-to-site causes (Table VII).

Measurement variability associated with the Mitutoyo QV404 jet area measurements for stage 2 varied from only

Table VII. Variability Components from Jet Area Data

Stage 2			Stage 7		
Variability source	Variability component	% of total	Variability source	Variability component	% of total
AVIS					
Inter-site	1.5×10^{-4}	89.1	Inter-site	2.3×10^{-6}	59.0
Replication	2.7×10^{-6}	1.6	Replication	5.4×10^{-9}	0.1
Residual component	1.6×10^{-5}	9.4	Residual component	1.6×10^{-6}	40.8
Total	1.70×10^{-4}	100.1	Total	3.81×10^{-6}	99.9
Mitutoyo					
Inter-site	2.1×10^{-6}	10.8	Inter-site	2.7×10^{-7}	19.5
Replication	UN	0.0	Replication	UN	0.0
Residual component	1.8×10^{-5}	89.2	Residual component	1.1×10^{-6}	80.5
Total	1.97×10^{-5}	100.0	Total	1.39×10^{-6}	100.0

UN undetectable

0.62% to 0.69% RSD (Table IV), with barely observable differences in absolute magnitude of the area measurements (Fig. 7a). Of the observed variation, 10.8% was associated with site-to-site causes (Table VII). As expected, the total variability was larger for stage 7 measurements, ranging from 1.98% to 2.30% RSD (Table IV), with a similar pattern in variability to that seen with stage 2 data (Fig. 7b). In this instance, 19.5% of the observed variation originated from site-to-site differences (Table VII).

Part 2. Measurement Accuracy

Individual observations from each participating laboratory for each element of the glass reticule and each of the ring gauges are presented as averages (n=30) in Table VIII together with the corresponding certified diameters (mm). By normalizing individual measurements against their nominal diameters collectively for each inspection system, an assessment of the overall accuracy and system bias could be made. Figure 8a shows that the 95% confidence intervals for all the systems are within 1% of their nominal values. This result indicates that the visual inspection systems evaluated are sufficiently accurate and precise for measuring diameters of impactor jets. A similar approach was used to assess measurement bias relative to the type of object calibration standard. While all instruments reported a marginally larger diameter for glass spots (bright aperture against a dark background) compared with an identically sized optically opaque chrome spot against a bright background (Fig. 8b), overall, the bias due to calibration artifact was <1%.

Evaluation of the individual reticule elements obtained at participating sites and expressed as percent certified diameter demonstrated that the measurements from the Mitutoyo, AVIS ('spots' only) and OGP instruments were within ±0.2% of the certified value for both 'spots' and 'dots', irrespective of size (Figs. 9a and 10a). In the case of the RAM Datastar and Omis II instruments, the magnitude of the reported diameter tended to increase with decreasing size for glass spots on a chrome background ('spots'; Fig. 9b), an effect that was repeatable in the case of the Omis II system in which a second series of measurements was attempted, in the worst case approaching +2.8% nominal diameter at the smallest size (0.254 mm diameter), comparable with the nominal jet size of stage 7 of the ACI. This trend was reversed when measuring chrome dots on a glass background, with the worst case (Omis II) approaching −1.5% of the certified value (Fig. 10b).

The measurement systems in general underestimated the diameter of the smallest ring gauge (no. 1, having a certified diameter of 1.00097 mm) as compared to the glass spot on chrome background of similar size (Fig. 11). In most instances, this bias was small, although in the worst case (Omis II), it amounted to −0.8% of the certified value.

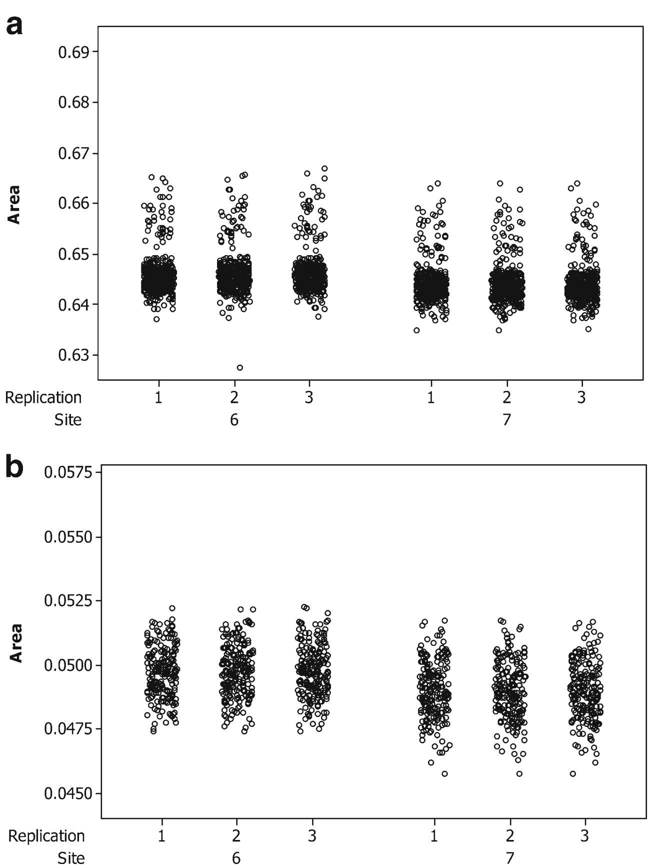

Fig. 7. Comparison of Mitutoyo QV404-measured jet areas for **a** stage 2 and **b** stage 7

DISCUSSION

Equipment Configuration

The optical inspection equipment (Table I) had many features in common. Each system comprised a CCD camera and lens that allowed focusing on an individual stage jet and image projection at the exit plane for analysis. The camera was mounted above a platform upon which an inverted stage was placed for inspection. The light source, which served to create an image on the CCD lens by transmitted light through the impactor stage jet undergoing mensuration, was located beneath the platform. Software controlled movement of the platform or camera to sequentially maneuver each jet beneath the camera for inspection and to process the acquired data. Closer inspection revealed important differences with regards to image focusing, image edge detection, and image analysis. Various degrees of technician interaction were required from preparation of the instrument for stage jet measurements through analysis of the collected image.

In the case of the AVIS, RAM Datastar, and Omis II instruments, focus and light levels were manually adjusted for one jet of the stage to obtain an optimal image in the CCD camera and these settings were maintained during subsequent measurements. However, for the AVIS equipment, the operator was required to identify the optimal lens position where the measured light intensity across the jet was uniform. Once these initial parameters were set, image collection and analysis of each jet on the stage were fully automated. Image analysis was accomplished for both RAM instruments by edge detection algorithms to measure image circumference in a fully automated manner, from which diameter was calculated. This algorithm calculated jet diameter based on a maximum of 500 points around the image circumference. The user had a choice of three algorithms (least squares fit, maximum inscribed fit, and minimum circumscribed circle fit) to best suit the application. The AVIS systems determined jet dimensions based only on image area, corresponding to the number of pixels illuminated in the camera, so that image diameter was calculated based on the diameter of a circle having the same area as the image. The Mitutoyo QV404 systems were fully automated, offering the capability for light adjustment and focus for each jet presented to the lens for measurement (a technician would only enter system settings prior to analysis, which were applied to all jet images collected for the stage) and were capable of completing measurements for an entire stage in about 15 min. These instruments also employed an edge detection algorithm to calculate jet diameter based on approximately 200 points around the circumference of the image, and in addition they measured image area based on the number of pixels illuminated in the camera. The OGP Smartscope was as automated as the QV404 system, but image analysis was limited to edge detection measurements with similar algorithms to those for the RAM systems. By contrast, the Mondo systems were fully manual in operation, which severely increases the time for stage analysis. Even with measurement of a restricted number of jets per stage, the process required about 45 min of analyst time to analyze one stage.

Calibration procedures used for the various optical inspection systems did vary in detail from site to site, but not sufficiently to warrant description here. Importantly, all participants made use of fully length-traceable test artifacts such as gauges (including ring gauges, gauge blocks, or in one instance, an independently calibrated (reference) ACI stage) or reticules as summarized in Table I.

Finally, it should be noted that magnification of the image through the lens with any of these systems was not a fixed parameter, but was dependent on the instrument configuration/design. For example, the Mitutoyo QV404 utilized two different lenses for magnification, depending on dimensions of image for analysis, to maintain an image that was greater than the minimum setting for the field of view. Image magnification for all instruments varied as part of the focusing process across the range of object sizes evaluated in this study.

Measurement Precision

The information gathered in this part of the study provided insight as to whether the different systems in use for CI stage mensuration are suitable, as well as indicating their relative precision with operators skilled in this practice. The data indicate that the reproducibility of site-to-site jet measurements is acceptable in relation to the established CI stage specification limits (13,14), based on both individual participant data (Fig. 2a and b and Tables II, III, and IV), and extreme overall values (Table V). This is an encouraging

Table VIII. Size Measurements Reported from Certified Standards

Reticule: chromium dots on glass								
Dot no.	Certified value (mm)	Mean measured diameter (mm; n=30)						
		QV404 (Site 7)	QV404 (Site 8)	Omis (Site 9)	Datastar (Site 8)		OGP Smart Scope (Site 9)	Omis Repeat (Site 9)
1	0.2540	0.2534	0.2536	0.2515	0.2535		0.2536	0.2505
2	0.3429	0.3424	0.3427	0.3404	0.3429		0.3426	0.3392
3	0.5330	0.5322	0.5326	0.5301	0.5331		0.5328	0.5290
4	0.7110	0.7102	0.7101	0.7078	0.7120		0.7098	0.7065
5	0.9140	0.9126	0.9132	0.9105	0.9152		0.9128	0.9091
6	1.8900	1.8883	1.8887	1.8807	1.8851		1.8887	1.8899
7	2.5500	2.5478	2.5486	2.5396	2.5464		2.5484	2.5548
8	4.5000	4.4981	NM	4.4824	NM		4.4936	4.5110
9	5.5000	5.4995	NM	5.4972	NM		5.4981	5.5010
Reticule: glass spot on chromium								
Spot no.	Certified value (mm)	Mean measured diameter (mm; n=30)						
		QV404 (Site 7)	QV404 (Site 8)	Omis (Site 9)	Datastar (Site 8)	AVIS (Site 1)	OGP Smart Scope (Site 9)	Omis Repeat (Site 9)
1	0.2539	0.2544	0.2535	0.2610	0.2555	0.2540	0.2542	0.2593
2	0.3429	0.3427	0.3426	0.3499	0.3452	0.3431	0.3433	0.3481
3	0.5331	0.5327	0.5325	0.5395	0.5367	0.5330	0.5333	0.5377
4	0.7111	0.7106	0.7100	0.7162	0.7161	0.7110	0.7113	0.7135
5	0.9140	0.9133	0.9131	0.9158	0.9204	0.9140	0.9145	0.9154
6	1.8901	1.8896	1.8895	1.8881	1.9003	1.8900	1.8920	1.8964
7	2.5500	2.5495	2.5494	2.5461	2.5617	2.5500	2.5536	2.5613
8	4.5000	4.5001	NM	4.4919	NM	4.5000	4.5036	4.5305
9	5.5000	5.5002	NM	5.5153	NM	5.5000	5.5029	5.5228
Ring gauges								
Gauge no.	Certified value (mm)	Mean measured diameter (mm; n=30)						
		QV404 (Site 7)	QV404 (Site 8)	Omis (Site 9)	Datastar (Site 8)	AVIS (Site 1)	OGP Smart Scope (Site 9)	Omis Repeat (Site 9)
1	1.0010	0.9993	0.9988	0.9934	1.0033	1.0000	0.9977	1.0017
2	2.4995	2.5006	2.5023	2.5023	2.5100	2.5000	2.4989	2.5102
3	4.5001	4.5000	NM	4.5074	NM	4.5000	4.5077	4.5175

NM not measured
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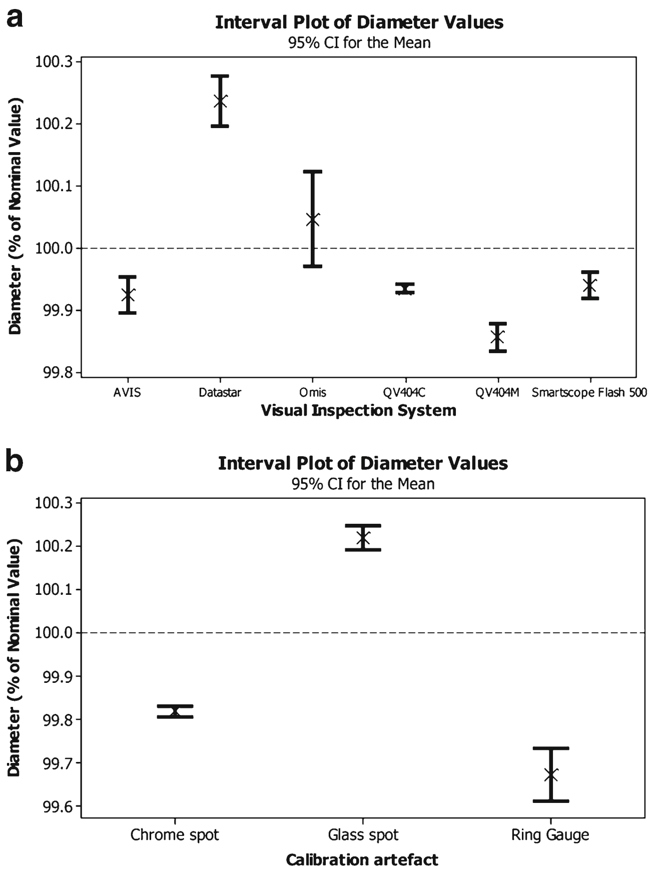

Fig. 8. Overall accuracy for the optical inspection systems by **a** optical inspection system and **b** nature of calibration artifact

outcome, given the differences in instrument design and degree of automation for these inspection systems (Table I).

Sources of data variability for a given optical inspection system were attributed as arising from both site-to-site and from replicate measurements of the same object, with remaining undefined causes of variability assigned to the residual component (Appendix). Of necessity the design of this investigation focused more on site-to-site variability than other aspects related to instrument design over which there is little external control. Interestingly, where comparisons between systems of the same type were possible at different locations, both inter-site variability and the residual component separately contributed more to the overall variability than the replication error (Tables VI and VII). Except for participant 6, who supplied data generated by both Mondo and Mitutoyo systems, the design of the precision study did not make it possible to compare the precision of different optical inspection systems at a particular site. However, given the small sample size, such observations are believed to be of limited value and are, therefore, not discussed further.

Inter-site variability is of particular interest, because the same make of optical measurement system could be used either by different organizations undertaking mensuration or by the same organization at different locations. In this context, comparative measurements made with the AVIS systems with both reference stages (Fig. 3a and b) demonstrated that replication and residual components were small in relation to the inter-site variability. This finding indicated good reproducibility existed at each of the organizations using this equipment, even if the absolute values from one participant did not match perfectly with those from the others. The total variability in jet diameter for stage 7, whether determined directly (Tables II and III) or computed from area measurements (Table IV), was larger than equivalent measures for stage 2. However, this variability is likely still acceptable given the smaller absolute size of the jets of stage 7.

It is an acknowledged limitation that an unknown portion of the overall imprecision observed for these instruments may have originated from the subjective judgment of the operator associated with manual focusing of the CCD camera, introducing small changes to the resulting image at each intervention. In this context, the absence of a detectable replication component to overall variability with the Mitutoyo systems is remarkable. In addition, measurements made with these more fully automated systems had less overall variability than the equivalent AVIS-derived results (compare Fig. 3a with Fig. 4a and Fig. 3b with Fig. 4b) As might be expected from manually operated systems, a larger spread in the individual measurements was evident with the Mondo inspection equipment (Fig. 5a and b). However, because of the small data set obtained from the Mondo system (compared to the automated measurement systems), the sample standard deviation may not accurately reflect the spread of the measurements (i.e., measurement uncertainty is higher in this case). It is important to note that this study was not intended nor powered to determine whether or not such

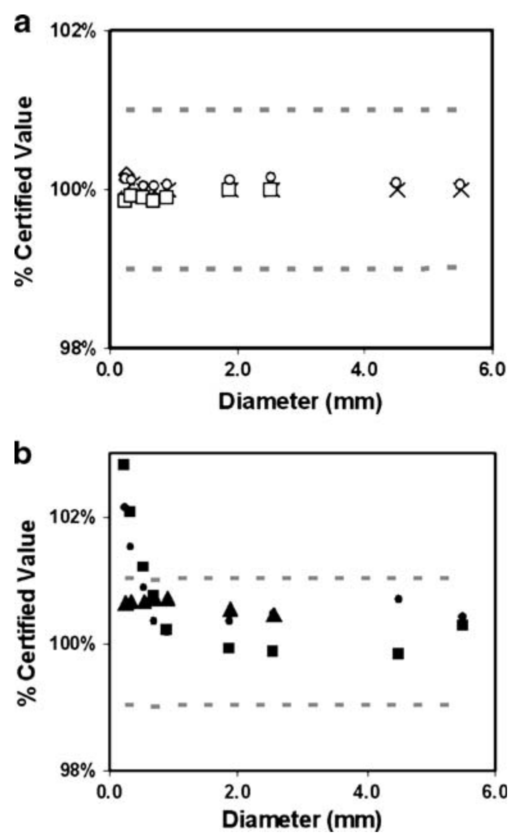

Fig. 9. Comparison of instrument accuracy for glass-on-chromium spots for certified reticule for **a** Mitutoyo site 7 (*open diamond*), site 8 (*open square*), OGP Smartscope (*open circle*), and AVIS site 1 (*multiplication symbol*) systems and **b** RAM Datastar (*close triangle*) and Omis II (initial (*close square*) and repeat (*close circle*)) systems (*discontinuous lines* represent ±1% of certified value). Used with permission from (7)

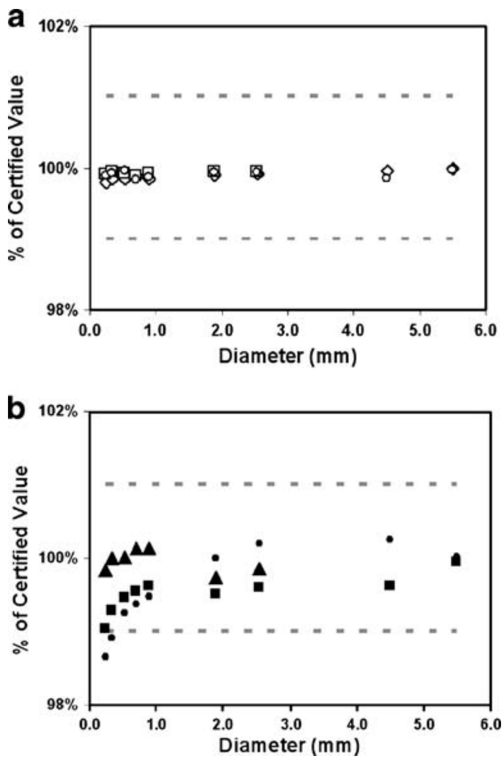

Fig. 10. Comparison of instrument accuracy for chromium-on-glass dots for certified reticule **a** Mitutoyo site 7 (*open diamond*), site 8 (*open square*), OGP Smartscope (*open circle*) systems and **b** RAM Datastar (*close triangle*) and Omis II (initial (*close square*) and repeat (*close circle*)) systems (*discontinuous lines* represent ±1% of certified value). Used with permission from (7)

differences were caused by controllable factors such as operator skill or originating from the instrumentation itself.

Finally, in the case of the AVIS instruments which only directly measure jet area, the higher magnitude of overall variability (RSD value) associated with jet diameter values computed from area measurements (Table II) compared with their equivalent values measured directly (Table IV) is readily explicable in terms of the relationship between the square of jet diameter and area.

Measurement Accuracy

The three different sizes of fully size-traceable ring gauge were intentionally selected as reference comparators since it is believed that the two-dimensional image projected from light striking each three-dimensional gauge aperture would include peripheral shadows making it more representative of the exit plane of a CI jet orifice than either the flat opaque or fully transparent circles of the reticule. Furthermore, it is likely that the jet depth profile, where microscopic but visible machining imperfections exist, could affect measurement process, thereby enhancing bias.

Each measurement system consistently reported a larger diameter for a spot (clear hole) of a given size compared with the equivalent opaque dot (Fig. 8a), and the magnitude of this bias increased as the size of the calibration spot was reduced. Nevertheless, under worst-case circumstances, the overall effect was small (<0.6% of the certified value) and is almost certainly attributable to the systematic allocation of partially lit pixels located along the image circumference either to the transparent aperture with the glass spots or to the transparent border adjacent to the chrome dots during analysis by the software of the different systems. The source of the larger bias observed with the RAM Omis II compared with the other systems when examining the smallest objects on the certified reticule is therefore almost certainly incorrect assignment of partially lit pixels by the Omis II image analysis software. It appears that pixels located at the periphery of the image of the opaque (dark) spot were incorrectly assigned to the illuminated region of the image. If true, this behavior would indicate that the image resolving power of these systems was close to the limit of capability.

The slight but observable underestimation of the size of the smallest ring gauge by most of the systems (Fig. 11) most likely originated from internal reflection of light in the ring gauge's three-dimensional profile that affects the lighted pixel intensity at the two-dimensional projected image. Any change in the pixel intensity at the plane for object analysis would have affected the measured diameter reported by the optical system. Nevertheless, it is likely that this form of bias is sufficiently small to be unimportant even for mensuration of stage 7 of the ACI where the smallest nominal jet diameter is 0.254 mm; however, it may become a concern for other CI designs if smaller jets are present.

CONCLUSIONS

The assessment of measurement precision using ACI reference stages 2 and 7 confirmed that all optical inspection systems evaluated provided measures of jet diameter that were within the specification limits defined in the European and US Pharmacopeias. Further analysis revealed that the precision of the automated systems was generally better than for the manually operated equipment (Mondo). Where between-site comparisons were possible, inter-site differences dominated the variability associated with the AVIS-based measurements but not for equivalent measurements made by the Mitutoyo automated systems. A repeatable size-related bias was evident with one of the diameter-based mensuration systems (RAM Omis II) in the accuracy assessment, which was attributed to its boundary recognition algorithm. In all cases, the overall measurement error for the evaluated stage mensuration systems appears to be better than 1% of nominal

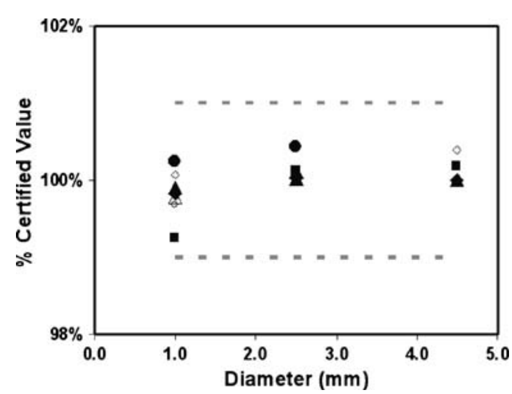

Fig. 11. Comparison of instrument accuracy of ring gauges for AVIS site 1 (*close triangle*), RAM Datastar (*close circle*) RAM Omis II (initial (*close square*) and repeat (*open diamond*)), Mitutoyo QV404 site 7 (*close diamond*) and site 8 (*open triangle*), and OGP Smart Scope (*open circle*) systems. *Discontinuous lines* represent ±1% of certified value

jet diameter, making all the techniques assessed suitable for confirming CI system suitability.

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APPENDIX: RATIONALE FOR FURTHER STATISTICAL ANALYSIS OF PRECISION DATA

The purpose of the precision study was to gain an understanding of likely variability associated with CI stage mensuration when a variety of optical inspection systems in current use measured the same stages 2 and 7 of the ACI that were circulated between sites in the round-robin exercise. Such information is helpful in understanding whether these systems are suitable for purpose such that the degree of precision available is adequate to enable compliance with specifications for CI stages that are published in the US and European Pharmacopeias (A1, A2).

The total measurement variability (error) reported in Tables VII (directly measured jet diameter) and VIII (jet diameter computed from measured jet area) can be broken down into the following origins by two-way ANOVA:

1. Site-to-site deviations in procedure (defined as the 'inter test site component)
2. Variability associated with repeated measurements of the same standard stage (defined as the 'replication' component)
3. The residual component of the analysis that includes factors such as operator and time (day) of measurement that were not analyzed for separately

In some instances, the same type of optical inspection system was used at different participant locations, making it possible to compare site-to-site variability. Except for site 6, which included data generated by both the Mondo and Mitutoyo systems, the design of the precision study did not make it possible to compare within-site precision of different optical systems.

REFERENCES

A1 European Directorate for the Quality of Medicines and Healthcare (EDQM). Section 2.9.18—Preparations for inhalation: aerodynamic assessment of fine particles. European Pharmacopeia 6th Edn., Strasbourg, France: Council of Europe; 2009. p. 287–300.

A2 United States Pharmacopeial Convention. (USP) Chapter 601—Physical tests and determinations: Aerosols. Rockville, MD: USP30-NF25; 2007. p. 220–240.

REFERENCES

1. United States Federal Drug Administration (FDA). Draft Guidance: Metered dose inhaler (MDI) and dry powder inhaler (DPI) drug products chemistry, manufacturing and controls documentation. Rockville MD, United States: Federal Drug Administration; 1998. Docket 98D-0997.
2. European Medicines Agency (EMEA). Guideline on the pharmaceutical quality of inhalation and nasal products. London, UK: European Medicines Agency; 2006. EMEA/CHMP/QWP/49313/2005 Final.
3. Marple VA, Liu BYH. Characteristics of laminar jet impactors. Environ Sci Technol. 1974;8:648–54.
4. Marple VA, Willeke K. Inertial impactors: theory, design and use. In: Liu BYH, editor. Fine particles. NY: Academic Press; 1976. p. 411–66.
5. Mitchell JP, Nagel MW. Cascade impactors for the size characterization of aerosols from medical inhalers; their uses and limitation. J Aerosol Med. 2003;16:341–76.
6. Nichols SC. Particle size distribution parameters using the next generation pharmaceutical impactor. In: Dalby RN, Byron PR, Peart J, Suman JD, Farr SJ, editors. Respiratory drug delivery-IX. River Grove: Davis Healthcare Int. Publ; 2004. p. 485–7.
7. Mitchell JP, Roberts DL, Chambers F, Aziz A, Shelton C, Nichols S. Cascade impactor calibration, mensuration and in-use qualification methods: a practical guide. In: Dalby RN, Byron PR, Peart J, Suman JD, Farr SJ, Young P. Respiratory Drug Delivery 2010, Davis Healthcare Int. Publ., River Grove, IL, (in press)
8. Berglund RN, Liu BYH. Generation of monodisperse aerosol standards. Environ Sci Technol. 1973;7:147–53.
9. Liu BYH, Pui DYH. A sub-micron aerosol standard and the primary calibration of the condensation nucleus counter. J Colloid Interface Sci. 1974;47:155–71.
10. Copley M. The importance of stage mensuration in cascade impaction. Pharm. Technol. Europe. Sept. 2008. Available at: http://www.ptemag.com/pharmtecheurope/analytical+equipment/The-importance-of-stage-mensuration-for-cascade-im/ArticleStandard/Article/detail/547892 (accessed 10 Jun 2009).
11. Christopher D, Curry P, Doub W *et al.* Considerations for the development and practice of cascade impaction testing including a mass balance failure investigation tree. J Aerosol Med. 2003;16:235–47.
12. European Directorate for the Quality of Medicines and Healthcare (EDQM). Section 2.9.18—Preparations for inhalation: aerodynamic assessment of fine particles. European Pharmacopeia 6th Edn., Strasbourg, France: Council of Europe; 2009, pp. 287–300.
13. United States Pharmacopeial Convention (USP) Chapter 601—physical tests and determinations: aerosols. Rockville, MD: USP30-NF25; 2007. pp. 220–40.
14. Roberts DL, Romay FJ. Relationship of stage mensuration data to the performance of new and used cascade impactors. J Aerosol Med. 2005;18:396–413.
15. Roberts DL. Theory of multi-nozzle impactor stages and the interpretation of stage mensuration data. Aerosol Sci Technol. 2009;43(11):1119–29.
16. Shelton CM. Comparison of optical systems for mensuration of 8-stage cascade impactor. In: Drug Delivery to the Lungs-15, London, UK: The Aerosol Society; 2004. pp. 133–6.
17. Mitchell JP. Good Practices of Qualifying Cascade Impactors (CIs): A Survey of Members of the European Pharmaceutical Aerosol Group (EPAG). Drug Delivery to the Lungs-16, London, UK: The Aerosol Society; 2005. p. 189–92.