

Validation of Semi-automated Flow Mediated Dilation Measurement In Healthy Volunteers

Short title: Semi-automated flow mediated dilation measurement

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Supplementary Material

Supplementary Table 1: Whole Cohort Characteristics

| | |
|---|------------------|
| Sex | |
| Male, n (%) | 18 (41.9) |
| Female, n (%) | 25 (58.1) |
| Smoking Status | |
| Non-smoker, n (%) | 39 (90.7) |
| Smoker, n (%) | 1 (2.3) |
| Ex-smoker, n (%) | 3 (7.0) |
| Diabetes | |
| No diabetes, n (%) | 41 (95.4) |
| Diabetes, n (%) | 2 (4.7) |
| Race | |
| Caucasian, n (%) | 35 (81.4) |
| Asian, n (%) | 6 (14.0) |
| Other, n (%) | 2 (4.7) |
| Cardiovascular Disease History | |
| No, n (%) | 43 (100) |
| Yes, n (%) | 0 (0) |
| Cancer History | |
| No, n (%) | 43 (100) |
| Yes, n (%) | 0 (0) |
| Time Between Study Visits (Days) | |
| Mean (SD) | 6.2 (4.2) |
| Median (min – max) | 6.0 (1 – 19) |
| Age (Years) | |
| Mean (SD) | 22.3 (4.2) |
| Median (min – max) | 22.0 (18 – 40) |
| Alcohol per week (units) | |
| Mean (SD) | 9.1 (7.0) |
| Median (min – max) | 7.0 (0.0 – 30.0) |
| Heart Rate (bpm) | |
| Mean (SD) | 66 (10) |
| Median (min-max) | 67 (49 – 84) |
| Systolic Blood Pressure (mmHg) | |
| Mean (SD) | 116 (13) |
| Median (min – max) | 114 (93 – 151) |
| Diastolic Blood Pressure (mmHg) | |
| Mean (SD) | 69 (8) |
| Median (min – max) | 68 (54 – 86) |

SD = standard deviation, min = minimum, max = maximum. A two-way t-test demonstrated there was no significant difference in characteristics between the supplement and main study cohort.

Supplementary Table 2: Reliability Statistics between different days

| Measurement | N | CV | ICCC | 95 % Confidence Interval | |
|---|----|--------|-------|--------------------------|-------------|
| | | | | Lower Bound | Upper Bound |
| Day 1 mean FMD + Day 2 measurement 1 FMD | 30 | 12.43% | 0.679 | 0.431 | 0.833 |

N = sample size, CV = Coefficient of Variation, ICC = intraclass correlation coefficient. Model utilised

= two-way mixed effects, absolute agreement based on single measures.

Supplementary Table 3: Sensitivity Analyses on elimination of diabetics and smokers

| | N | ICCC | 95% Confidence Interval | |
|-------------------------------|----|-------|-------------------------|-------------|
| | | | Lower Bound | Upper Bound |
| Day 1 Measurements | 30 | 0.810 | 0.638 | 0.905 |

ICCC = intraclass correlation coefficient, N = sample size. Sensitivity analyses on elimination of one diabetic and one smoker

Supplementary Table 4: Subgroup Analyses of Sex, systolic blood pressure and diastolic blood

pressure

| Variable | | N | Measurement 1: Mean FMD % ± SD | Measurement 2: Mean FMD % ± SD | ICC | 95% Confidence Interval | |
|---|---------|----|--------------------------------|--------------------------------|-------|-------------------------|-------------|
| | | | | | | Lower Bound | Upper Bound |
| Sex | Male | 13 | 12.06 ± 5.17 | 12.61 ± 4.53 | 0.839 | 0.562 | 0.948 |
| | Female | 19 | 12.33 ± 2.83 | 12.29 ± 2.96 | 0.786 | 0.522 | 0.912 |
| Systolic Blood Pressure Median = 115 | <Median | 16 | 12.08 ± 2.69 | 12.41 ± 3.14 | 0.744 | 0.425 | 0.899 |
| | >Median | 16 | 12.35 ± 4.85 | 12.42 ± 4.10 | 0.856 | 0.634 | 0.947 |
| Diastolic Blood Pressure Median = 68 | <Median | 16 | 12.12 ± 3.02 | 12.41 ± 3.24 | 0.737 | 0.376 | 0.903 |
| | >Median | 16 | 12.27 ± 4.74 | 12.34 ± 4.14 | 0.859 | 0.641 | 0.949 |

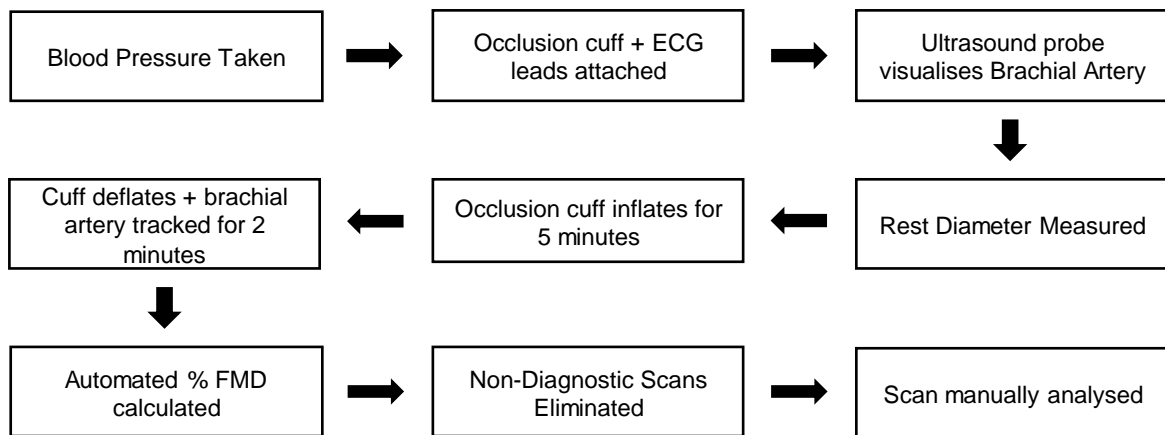
N = sample size, FMD = Flow Mediated Dilatation, ICC = intraclass correlation coefficient. Subgroup analyses of sex, systolic blood pressure and diastolic blood pressure. Model utilised = two-way mixed effects, absolute agreement based on single measures.

Supplementary Table 5: Supplementary Reproducibility Statistics

| Measurement Method | Variable | Within or Between Day Reproducibility | Bias | SD | 95% Limits of Agreement | |
|--------------------|---------------|---------------------------------------|------------|----------|-------------------------|-----------------|
| | | | | | Lower Limit (%) | Upper Limit (%) |
| Automatic | FMD | Between | -1.151% | 10.204% | -21.14 | 18.84 |
| | Rest Diameter | Within | 0.033 mm | 0.386 mm | -0.727 | 0.793 |
| | | Between | -0.0537 mm | 0.40 mm | -0.838 | 0.730 |
| | Max Diameter | Within | 0.0375 mm | 0.423 mm | -0.786 | 0.861 |
| Between | | -0.1137 mm | 0.493 mm | -1.05 | 0.823 | |
| Manual | FMD | Between | -0.597% | 3.241% | -6.947 | 5.753 |
| | Rest Diameter | Within | -0.012 mm | 0.361 mm | -0.720 | 0.696 |
| | | Between | -0.034 mm | 0.389 mm | -0.796 | 0.728 |
| | Max Diameter | Within | -0.0263 mm | 0.405 mm | -0.820 | 0.768 |
| Between | | -0.0627 mm | 0.408 mm | -0.879 | 0.753 | |

FMD = flow mediated dilation, SD = standard deviation, max = maximum

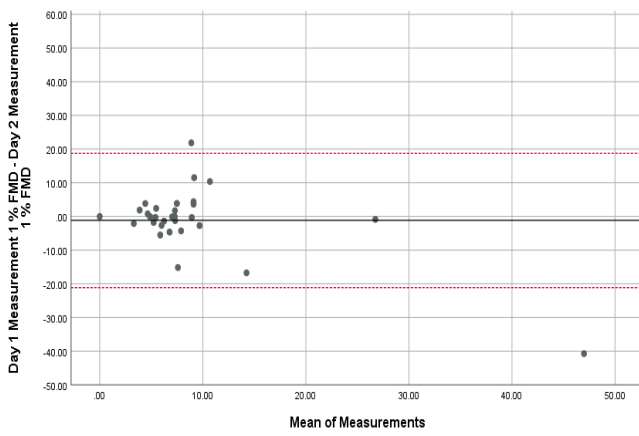
Supplementary Figure 1: FMD Protocol



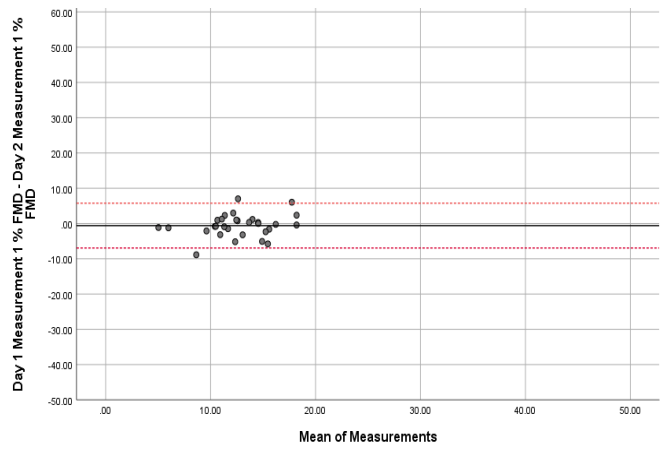
% FMD = percentage flow mediated dilation.

Supplementary Figure 2: Bland-Altman Plots of % FMD

A

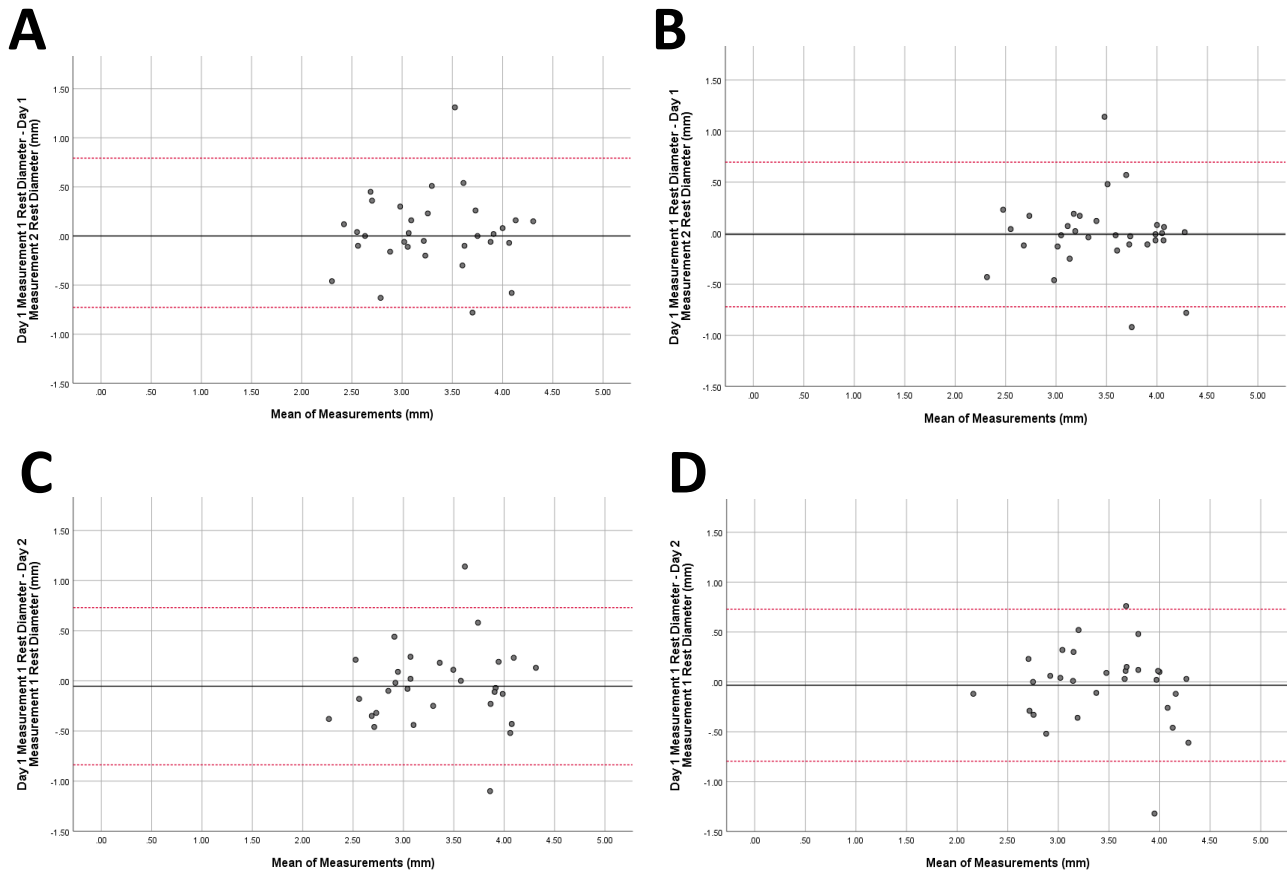


B



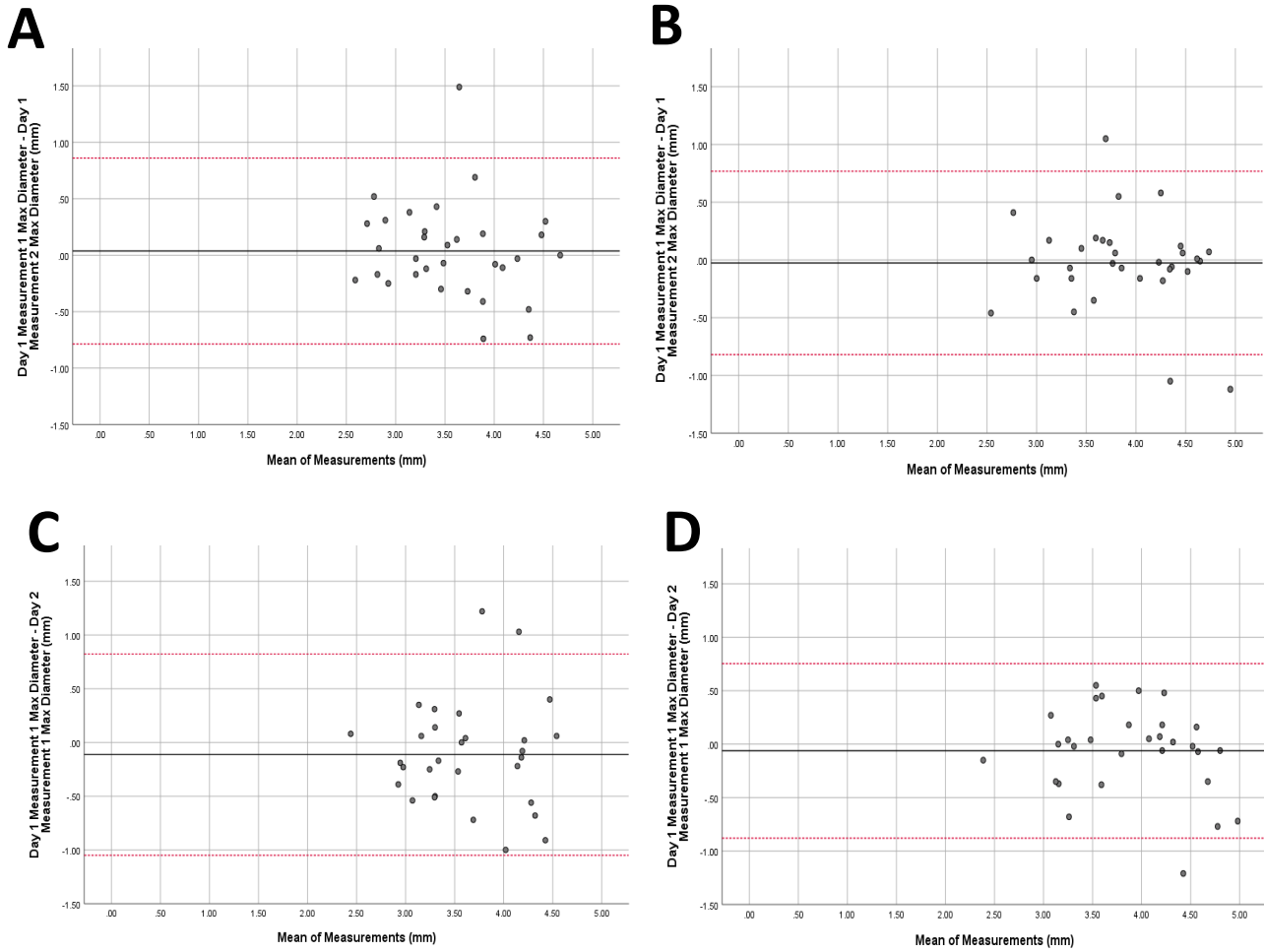
Bland-Altman plots of % FMD readings conducted on separate days for manual and automated analysis. A: Automated results FMD on separate days, B: Manual results FMD on separate days, Broken red lines = 95% limits of agreement, % FMD = percentage flow mediated dilation

Supplementary Figure 3: Bland-Altman Plots of Rest Diameters



Bland-Altman plots of rest diameters for manual and automated analysis. A: Automated results rest diameter on the same day, B: Manual Results rest diameter on the same day, C: Automated results rest diameter on separate days, D: Manual results rest diameter on separate days, Broken red lines = 95% limits of agreement, % FMD = percentage flow mediated dilation

Supplementary Figure 4: Bland-Altman Plots of Maximum Diameters



Bland-Altman plots of maximum diameters for manual and automated analysis. A: Automated results maximum diameters on the same day, B: Manual results maximum diameters on the same day, C: Automated results maximum diameters on separate days, D: Manual results maximum diameters on separate days, Broken red lines = 95% limits of agreement, % FMD = percentage flow mediated dilation.