# ANGIODEFENDER: Clinical validation studies

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## 1. METHOD REPRODUCIBILITY

### 1.1. Japan (2009): Flow-mediated dilation (FMD) reproducibility testing using the AngioDefender prototype device, AngioEF-3000

In February 2009, the AngioDefender prototype device (AngioEF-3000™; Angiologix) underwent reproducibility testing in Japan, involving 23 repeat measurements on 21 volunteer subjects with a range of cardiovascular disease (CVD) risk factors. No spontaneous adverse device effects (ADEs) were reported. The %FMD of the brachial artery (BA) was measured twice for the same individual, 1-4 hours apart, on the same day. Mean %FMD values ranged from 8% to 36%. Satisfactory %FMD reproducibility was demonstrated, with a single measures intraclass correlation coefficient (*r*) of 0.62 when all 23 repeat measurements were incorporated and an *r* of 0.73 when the outlying data of 1 of the repeat measurements was excluded (indicated as red line in **Figure 1**).

**The test runs were not statistically different** when the data (22 repeat measurements) was analyzed using the non-parametric Wilcoxon test for paired samples: 2-tailed probability, p=0.074

Coefficient of variation from 22 duplicate measurements: 20.6%

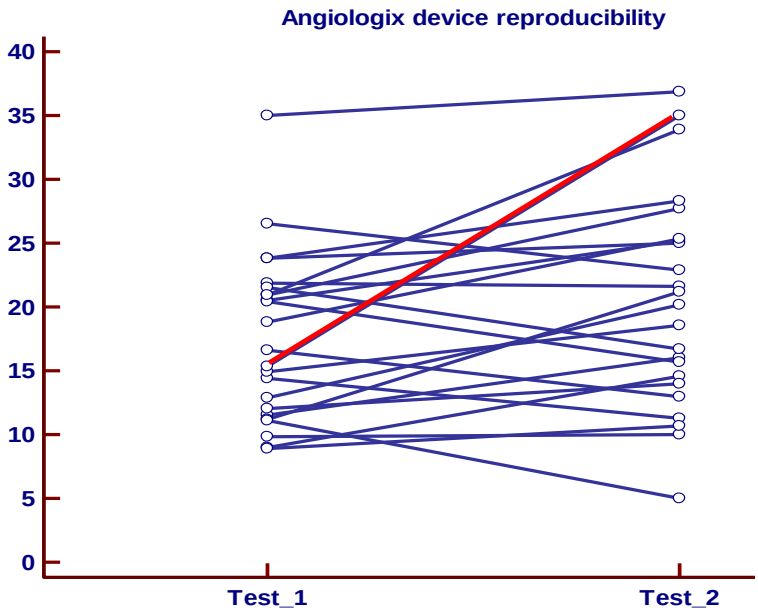



**Figure 1.** Dot-and-Line diagram illustrating the degree of absolute agreement among 23 repeat %FMD measurements

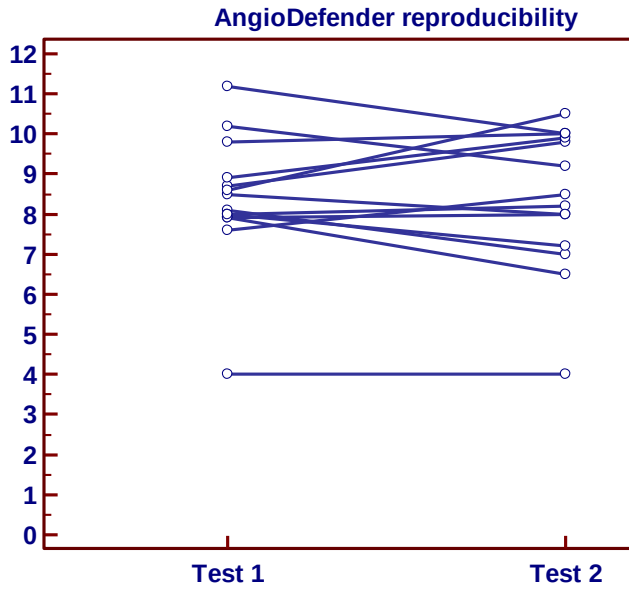
### 1.2. US (2011): FMD reproducibility testing using the AngioDefender device

In July 2011, the AngioDefender device underwent reproducibility testing in the US, involving 14 repeat measurements on 8 volunteer subjects with a range of CVD risk factors. No spontaneous ADEs were reported. Maximum %FMDmax (%FMDmax) of the BA was measured twice for the same individual, on separate days. Mean %FMDmax values ranged from 4% to 11%. Satisfactory %FMDmax reproducibility was demonstrated, with a single measures intraclass correlation coefficient (*r*) of 0.84.

**The test runs were not statistically different** when the data (14 repeat measurements) was analyzed using the non-parametric Wilcoxon test for paired samples: 2-tailed probability, p=0.84 (**Figure 2**)

Coefficient of variation from 14 duplicate measurements: 8.2%

**Figure 2.** Dot-and-Line diagram illustrating the degree of absolute agreement among 14 repeat %FMDmax measurements




## 2. COMPARISON TO BRACHIAL ARTERY ULTRASOUND IMAGING (BAUI) FOR DETERMINING %FMD

### 2.1. Russian Federation (2010)

In 2010, determination of %FMD of the BA using the AngioEF-3000 prototype device (index test) was compared to that obtained using the gold-standard BAUI technique (reference test). The hypothesis was that the AngioDefender method would produce the same %FMD values as BAUI while not requiring expensive vascular ultrasound equipment and the associated requisite operator expertise. Each of 22 volunteer subjects with a range of CVD risk factors underwent FMD testing using both procedures, separated by a 1-hour interval. The order in which the tests were performed was randomly selected. No spontaneous ADEs were reported.

Pearson correlation coefficient = 0.84 ($p<0.0001$)

**Figure 2** illustrates a Passing and Bablok regression plot of the data, showing close matching of the regression line with the line-of-identity. In light of the fact that this analysis demonstrated that the 95% CI of the Y-intercept contains zero (-1.6 to 1.5), the 95% CI for the regression line contains 1.0 (0.63 to 1.5), and there was no significant deviation from linearity ($p=0.78$), one can conclude that **the 2 techniques are statistically equivalent as methods for quantifying %FMD of the BA.**

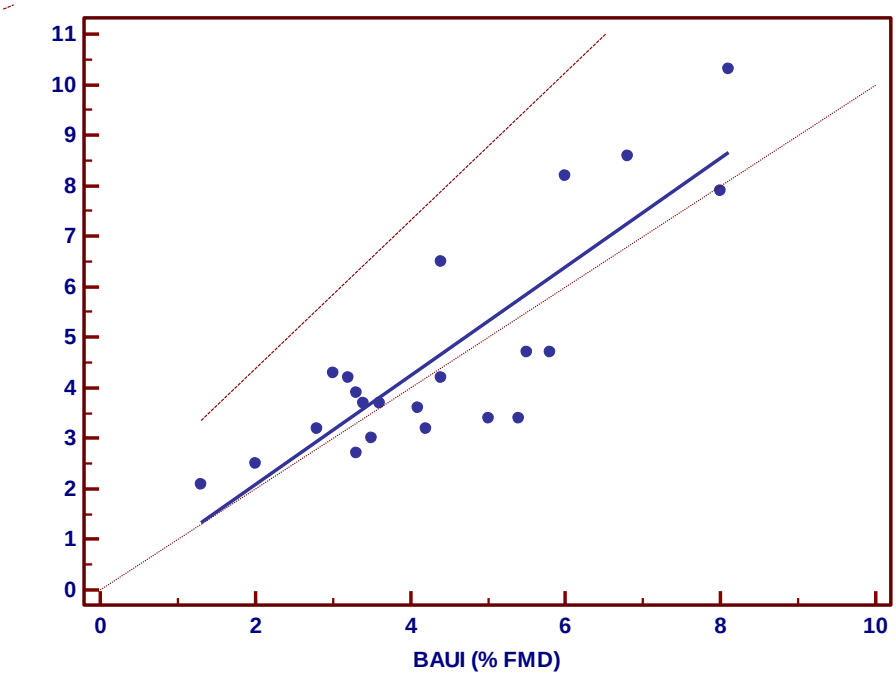



**Figure 2.** Passing and Bablok regression plot of Russian data showing statistical equivalence of the 2 techniques (n=22)

## 2.2. India (2011)

To obtain evidence that AngioDefender was able to achieve %FMD values that approximated those obtained using BAUI, a study (Protocol A-102-2) was conducted in 2011 by Dr. Ravi Kasliwal (Medanta Heart Institute) in New Delhi, India. The protocol was approved by all relevant regulatory bodies in India. Each of 33 volunteers underwent FMD testing using BAUI and AngioDefender. The 2 tests were performed on consecutive days for each individual. Whereas AngioDefender automatically provided a maximum %FMD (%FMDmax) value, the highest value obtained among 3 post-cuff release times (60, 90, and 120 seconds) when employing BAUI was considered the %FMDmax value for comparison. Results were analogous to those previously obtained in Russia, indicating that **AngioDefender and BAUI are statistically equivalent methods for quantifying %FMD of the BA** (**Figure 3**).

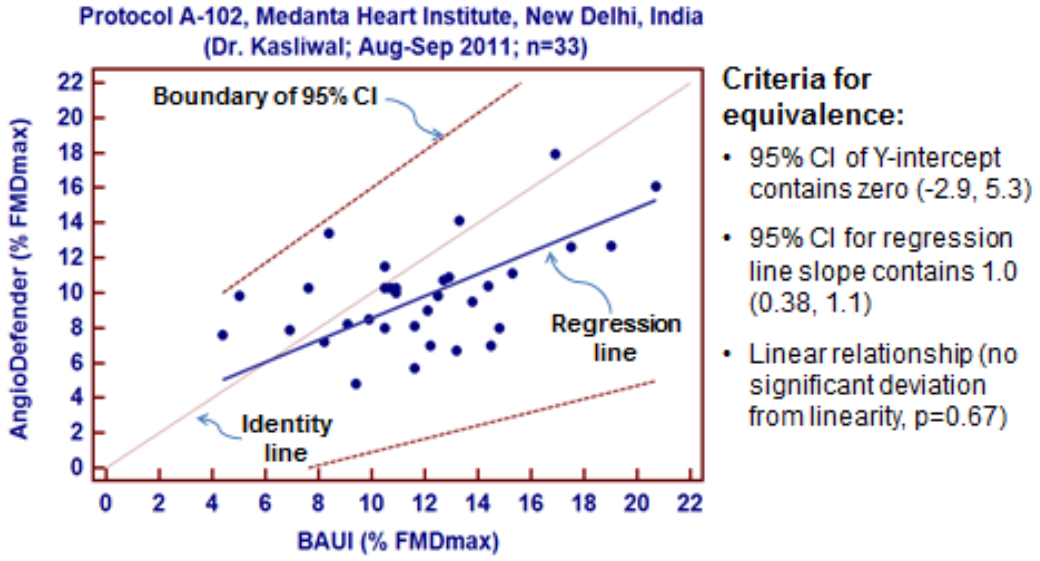



**Figure 3.** Passing and Bablok regression plot showing statistical equivalence of the 2 techniques (n=33)

## 3. RELATIONSHIP OF ANGIODEFENDER FMD VALUES TO CVD RISK FACTORS (India, 2011)

To illustrate the potential for AngioDefender to identify individuals at increased risk for future CV events despite not evidencing traditional CVD risk factors, a study (A-103-1) was conducted in May 2011 by Dr. SS Iyanger (Manipal Heart Institute) in Bangalore, India using the AngioEF-3000 device. The study enrolled 25 individuals with and without a variety of known CVD risks factors (**Figure 4**)

Using <10% FMD as a cut-off value indicating an elevated risk for future CVD, the results support a conclusion that the AngioDefender method for measuring %FMD of the BA is able to identify individuals that may have a significantly elevated risk for future CVD, but who would have been missed if one simply considered conventional blood pressure and diabetes status.

| Age | Gender | Diabetes | BP | BP Risk Range (Age Adjsted) | %FMD | AngioDefender CVD Risk Score |
|---|---|---|---|---|---|---|
| 32 | Male | n | 123/75 | Normal | 6.4 | Higher Medium CVD Risk |
| 39 | Male | n | 132/75 | Normal | 8.7 | Medium CVD Risk |
| 32 | Male | n | 127/72 | Normal | 11.4 | Low CVD Risk |
| 55 | Male | y | 116/71 | Normal | 9.6 | Medium CVD Risk |
| 46* | Male | n | 122/77 | Normal | 7.4 | Higher Medium CVD Risk |
| 46* | Male | y | 128/85 | Normal | 8.7 | Medium CVD Risk |
| 29 | Male | y | 110/63 | Normal | 20.2 | Low CVD Risk |
| 26 | Male | n | 128/79 | Normal | 4.2 | High CVD Risk |
| 34 | Female | n | 113/67 | Normal | 7.5 | Medium CVD Risk |
| 64 | Male | y | 129/86 | Normal | 4.7 | High CVD Risk |
| 27 | Female | n | 120/69 | Normal | 7.5 | Medium CVD Risk |
| 28 | Female | n | 123/72 | Normal | 7.3 | Higher Medium CVD Risk |
| 30 | Female | n | 121/70 | Normal | 9 | Medium CVD Risk |
| 21 | Female | n | 106/71 | Normal | 7.5 | Medium CVD Risk |
| 54 | Male | n | 127/87 | Normal | 8.1 | Medium CVD Risk |
| 33* | Male | y | 124/80 | Normal | 6.6 | Higher Medium CVD Risk |
| 33* | Male | y | 131/83 | Normal | 6 | Higher Medium CVD Risk |
| 38 | Male | y | 129/81 | Normal | 11.2 | Low CVD Risk |
| 51 | Female | y | 139/86 | Bordeline Normal | 13.8 | Low CVD Risk |
| 56 | Male | y | 132/80 | Bordeline Normal | 6.8 | Higher Medium CVD Risk |
| 59 | Female | n | 140/83 | Boderline Normal | 5.3 | High CVD Risk |
| 46 | Male | n | 133/79 | Boderline Normal | 6.1 | Higher Medium CVD Risk |
| 46 | Female | n | 151/97 | High | 10.2 | Low CVD Risk |
| 27 | Male | y | 140/91 | High | 9.4 | Medium CVD Risk |
| 68 | Male | y | 157/96 | High | 7.4 | Medium CVD Risk |
| 63 | Male | y | 157/91 | High | 5.8 | High CVD Risk |
| 48 | Female | n | 187/112 | High | 7.9 | Medium CVD Risk |

**Figure 4.** %FMD vs CVD risk factors