

## Why We Use the MMP-9 Reference Range We Use

One might ask why providers trained in the Shoemaker protocol do not use the published reference ranges of LabCorp and Quest when interpreting MMP-9 results? The answer is simple. The currently published results for MMP-9 are not correct. They are not consistent with the worldwide peer-reviewed published literature. They are not even consistent with other ranges for MMP-9 developed by Quest and LabCorp with known data! I was once asked at deposition if using other-than-published reference values was medical malpractice? I responded that, to know what I know, it would be malpractice to still use the currently published Quest and LabCorp reference ranges. I will explain.

LabCorp bought the Esoterix lab which developed the ELISA assay for MMP-9 and runs the assay to this day. Dr. Ritchie Shoemaker asked the Baltimore Quest lab to also run the assay. They agreed to if Dr. Shoemaker could provide case MMP-9 blood samples to ensure validation of the Esoterix test and control blood samples to define reference ranges. Dr. Shoemaker did so. The initial serum reference range of 85-332 ng/mL for Quest was established by William Meyer, PhD, MT, Technical Director of the Baltimore lab, in 2004, using Dr. Shoemaker's provided samples.

The history above defines how the original range was established, yet, the reference range LabCorp currently uses is 0-983 ng/mL. Quest, in the United States, outsources their MMP-9 samples to the same laboratory (Esoterix). Quest uses the reference range of 0-900 ng/mL of serum, even though it is using the same laboratory for testing as LabCorp. How did these very broad ranges come about?

In other parts of the world, the reference ranges for MMP-9 are significantly different. In Europe, Mortlock<sup>1</sup> found the serum reference range of MMP-9 for women to be 14.3 - 34.6 ng/mL and 19.8 - 99.5 ng/mL in men. Mortlock employed the standard formula used for determining reference ranges: the mean of healthy control values  $\pm$  2 standard deviations.<sup>2</sup> In other words, for women, the mean serum MMP-9 was 24.45 and the standard deviation was 5.075 ng/mL while for men, the mean value was 59.65 ng/mL with a standard deviation (SD) of 19.925.

The lower limit of the reference range was calculated by subtracting 2 times the SD from the mean, while the upper limit was obtained by adding 2 times the SD to the mean. For instance, taking the women's mean of 24.45 and subtracting 2 times the SD ( $2 \times 5.075 = 10.15$ ) gives the equation  $24.45 - 10.15 = 14.3$ , the lower limit. The upper limit is  $24.45 + 10.15 = 34.6$  ng/mL. The  $\pm 2 \times$  SD is used because the Central Limit Theorem in statistics suggests in a population with a normal distribution, roughly 95% of all samples from healthy control patients will be found in the interval between 2 SD below the mean and 2 SD above the mean. As such, the lower 2.5% and upper 2.5% tails from the "Bell-shaped curve" are considered abnormal. A more precise way to state this is that those results in these tails are, statistically speaking, much more likely to

be abnormal than those results falling within the 95% (Figure 1). That's what I was taught in medical school and that continues to be the standard for defining reference ranges.<sup>2</sup>

### “Bell-shaped Curve” and Distribution of Values Related to Standard Deviation

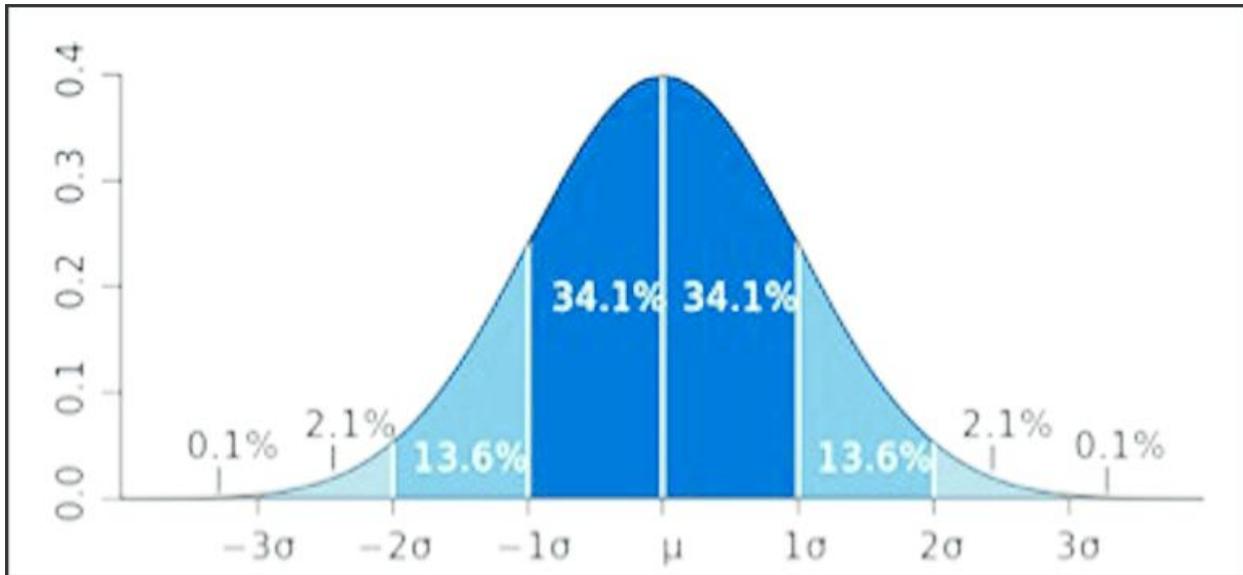


Figure 1

An idealized frequency distribution of values based on increments of standard deviation ( $\sigma$ ) above and below the mean ( $\mu$ )

Mortlock obtained these results evaluating 1250 controls and using an ELISA test kit made in London. Both these results included a 95% confidence limit (CL). At least 120 data points from controls are needed to insure 95% CL. Mortlock had 1250 data points.

Mortlock went on to say that, "During the primary validation of the MMP-9 assay at Q<sup>2</sup> Solutions (formerly Quest Diagnostics) a reference range had been assigned based on the manufacturer's recommendation (13-105 ng/mL) irrespective of gender." These numbers, generated from Quest, correlated well with the reference ranges Mortlock calculated.

Also in Europe, Mroczko<sup>3</sup> et al., showed 70 healthy controls had a median level of MMP-9 of 391 ng/mL. This was statistically different than colorectal cancer and colorectal adenoma patients with elevated MMP-9 medians of 530 ng/mL and 518 ng/mL, respectively.

In Japan, Iizasa<sup>4</sup> et. al, performed a plasma MMP-9 assay, using ELISA, on 138 healthy controls yielding a mean of 36.3 with SD of 13.2 ng/mL. This would create a reference range of 9.9 - 62.7 ng/mL. I am not aware of any other peer-reviewed and published studies reporting MMP-9 levels in healthy controls.

After releasing the MMP-9 test, and for years after, the Quest/LabCorp/Esoterix reference range for MMP-9 was 85-332 ng/mL. This would suggest a mean of 208.5 with SD of 61.75 ng/mL. After LabCorp/Esoterix changed the MMP-9 reference range, without a change in testing methodology, to 0-983 ng/mL, Dr. Shoemaker protested and, in 2011, submitted ~2500 MMP-9 samples to Dr. Dorothy Adcock, then and now the Director of Esoterix Coagulation Lab in Aurora, Colorado. As a result of these samples, for Dr. Shoemaker alone, using a special, private code, LabCorp and Esoterix generate results which use 64-386 ng/mL for MMP-9 results. I have seen the e-mail trail between Dr. Adcock and Dr. Shoemaker as well as sample MMP-9 result pages representing both the LabCorp and Esoterix labs, documenting this range, which Dr. Adcock had created for Dr. Shoemaker. For whatever reason, everyone else ordering an MMP-9 gets the 0-983 ng/mL range from LabCorp and Esoterix. If from Quest, the reference range reported is 0-900 ng/mL (Table 1).

Compilation of Various Published MMP-9 Reference Ranges (ng/mL)

Source	Mean	Standard Deviation	Lower Limit	Upper Limit
Mortlock <sup>1</sup> - women	24.45	5.075	14.3	34.6
Mortlock <sup>1</sup> - men	59.65	19.925	19.8	99.5
Mroczko <sup>3</sup>	391 <sup>a</sup>	-	-	<480
Iizasa <sup>4</sup>	36.3	13.2	9.9	62.7
Q <sup>2</sup> - manufacturer	59	23	13	105
Quest - original	208.5	61.75	85	332
LabCorp - Shoemaker	225	80.5	64	386
LabCorp - current	491.5	≥245.75	0	983
Quest - current	450	≥225	0	900

Table 1

The table compiles the published MMP-9 ranges of various sources including the means, standard deviations, upper limits of normal and lower limits of normal. Some of these values are reported, some calculated using the standard formula of Mean  $\pm$  2SD. Q<sup>2</sup> – manufacturer are the manufacturer's recommended reference numbers. Quest – original are the values from the 2004 reference range established by Dr. William Meyer. LabCorp – Shoemaker is the range established by Dr. Dorothy Adcock for Dr. Shoemaker in 2011. LabCorp – current, and Quest – current, are the ranges these companies currently publish. Note, the standard deviations for LabCorp – current, and Quest – current, could be larger than indicated and cannot be determined without data from these companies because the lower limits of normal are reported as zero (0).

<sup>a</sup> Median, not mean

What does all this mean? First, we must understand the proper context. Humans who are normal and healthy have a certain amount of MMP-9 in their blood. The amount may be somewhat different in each healthy person, but as with most blood tests, like sodium or calcium, the level falls into a narrowly defined range which can be evaluated statistically. Laboratories draw the blood of many healthy people and do that statistical analysis to determine the normal reference ranges of MMP-9 and many other analytes. The amount of MMP-9 in healthy humans has not changed. It has not tripled, as current LabCorp and Quest statistics would suggest. Humans have not, all of the sudden, developed a new normal range whereby nearly 3 times as much MMP-9 as previous is now acceptable. THIS, the actual amount of MMP-9 in healthy humans, is the baseline; the statistics are not the baseline but rather an analysis of measurements of the baseline. Further, while healthy human levels of MMP-9 have not changed, neither has the methodology by which Esoterix tests MMP-9 changed.

What changed is the way the reference range was calculated. The initial range of 85-332 ng/mL was calculated using the standard technique of measuring from at least 120 healthy controls, calculating the mean and SD and using the formulas of the mean minus 2 x SD as the lower limit and the mean plus 2 x SD as the upper limit. What changed is that the results of controls AND cases (sick people) apparently were pooled together. The statistics were run again, now giving a much higher standard deviation (now at least 225 ng/mL, or >3.5 times the previous highest SD, which also was from Esoterix), leading to much wider reference ranges. The SD are greater because the dispersion of data is greater. This is because controls, with normal values of MMP-9, and ill persons with elevated (by previously published reference ranges) were pooled into the same statistical analysis. As such, the "new and improved" reference ranges are useless and even dangerous. This is why I reject them (Figure 2).

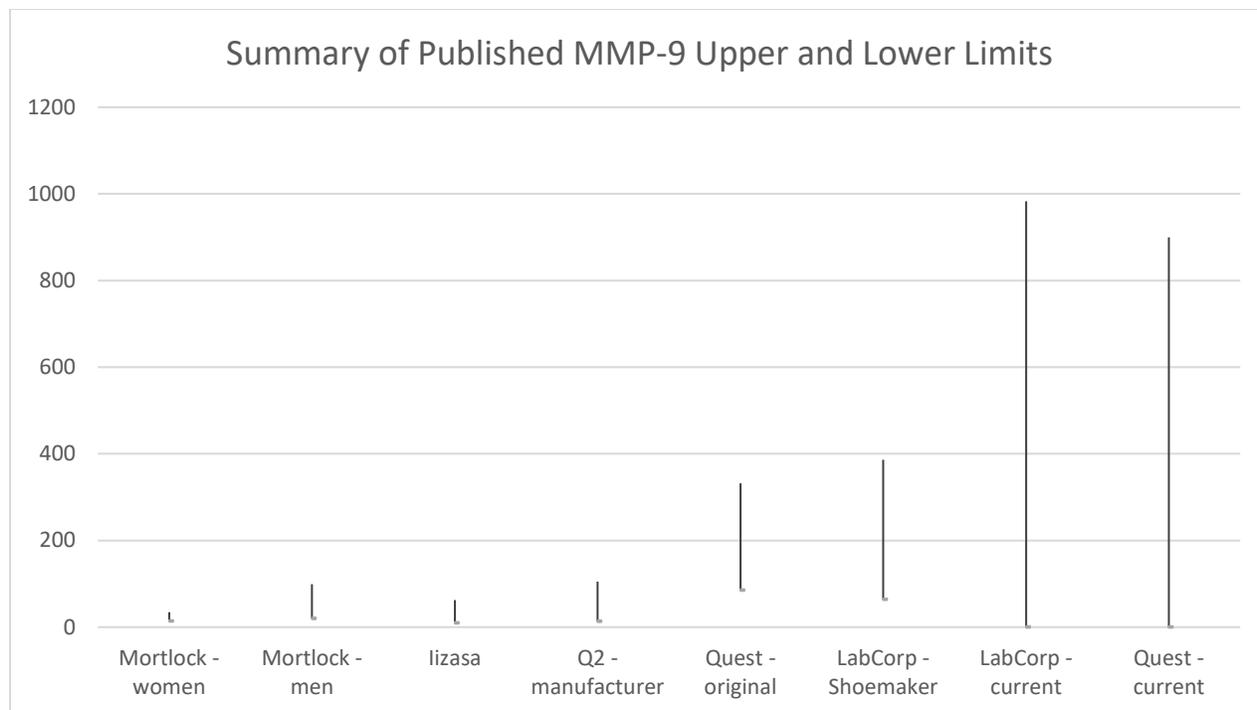


Figure 2

Graphic comparison showing lower limits of normal and upper limits of normal of several published reference ranges for MMP-9.

If Dr. Dorothy Adcock, Director of LabCorp's Esoterix Coagulation Lab in Aurora, Colorado, states that the range for Dr. Shoemaker is 64 – 386 ng/mL, the old Quest/LabCorp/Esoterix range is 85-332 ng/mL, 2 peer reviewed and published studies show the upper limit of MMP-9 normal is <100 ng/mL, and Quest in Europe also used a maximum of 105 ng/mL, why would anyone with this knowledge accept a reference range of 0-900 or 0-983 ng/mL? The truth is, they would not. I have that knowledge and I reject the bogus reference ranges. I continue to use Quest/LabCorp/Esoterix's previous range of 85-332 ng/mL. The reader now has that knowledge too.

<sup>1</sup> Mortlock S. Matrix metalloproteinase-9: Defining a normal reference range. Sch. J. App. Med. Sci. 2016;4(11D):4146-4150.

<sup>2</sup> Jones G, Barker A. Reference Intervals. Clin. Biochem Rev. 2008 Aug; 29(Suppl 1):S93-S97.

<sup>3</sup> Mroczko B, Groblewska M, Okulczyk B, Kedra B, Szmitkowski M. The diagnostic value of matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of matrix metalloproteinases 1 (TIMP-1) determination in the sera of colorectal adenoma cancer patients. Int J colorectal Dis. 2010;25(10):1177-84.

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<sup>4</sup> Iizasa T, Fujisawa T, Suzuki M, Motohashi S-I, Yasufuku K, Yasukawa T, Baba M, Shiba M. Elevated levels of circulating plasma matrix metalloproteinase 9 in non-small cell lung cancer patients. *Clinical Cancer Research*. 1999 Jan;5(1):149-53.