



# Core Lab Analysis Validation in Cardiovascular Clinical Trials

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In virtually all therapeutic areas imaging techniques are being used for the diagnosis and monitoring of disease in patients, as well as for the evaluation of the efficacy of a particular treatment. In clinical routine, this is mostly done by visual interpretation of the images. Based on the experience and expertise of the specialist, and eventually in combination with other non-imaging observations, the optimal treatment is chosen. In support of the visual interpretation, software tools integrated in offline applications or applied online during the imaging procedures can be used. Such tools can vary from a simple calliper for point-to-point measurements, to advanced 3D image-processing techniques for time varying images, such as in cardiovascular applications. It is not hard to imagine that with the large variety of acquisition equipment and post-processing software tools available, plus the variability due to manual choices and interactions, outcomes of these measurements can significantly differ. For this reason, if for example in clinical trials enrolment criteria or study outcome data are based on imaging measurements, variability usually increases with the number of investigative sites participating in a clinical trial.

This article will explore several aspects related to core lab validation based on our expertise and experience in the development of analytical software packages for clinical and research applications, and in applying such software in central offline core lab analyses. The application of quantitative coronary arteriography (QCA) in cardiovascular clinical trials will be taken as an example.

## HISTORY

One of the very first cardiovascular imaging applications to emerge was X-ray left ventriculography. In the 1960s and 1970s, this resulted in the development of software tools for the segmentation of the left ventricular contours and the quantification of the regional left ventricular function. All still required significant user interaction to make the appropriate corrections to the detected contours. In the late 1970s, the quantification of the progression and regression of coronary artery disease became possible with the introduction of quantitative coronary arteriography (QCA). Indeed, as of today, QCA is the most accurate and reliable technique to assess changes in lumen sizes within the entire coronary and peripheral vasculature over time. Since 1999, the fourth generation of QCA analysis programmes has become available, characterised by a broad range of specific analysis tools (such as for brachytherapy, drug eluting stent analysis and bifurcation analysis), simplified portability to DICOM viewers, and connectivity to PACS systems. However, it is still the experience and consistency of the user in applying these tools that determines the final accuracy of the QCA analysis outcome. For example, in interventional cardiology it is known that patient enrolment significantly decreases when online QCA is used to assess vessel size and severity of atherosclerotic plaques and thus determine whether these meet the inclusion and exclusion criteria. It appears that visual estimation is indeed much less reliable and subject to large variability in interpretation. For this reason, core labs are used to provide an independent, reliable and objective analysis in clinical trials. But even though their importance is obvious, there is no clear international guidance on the comparison of QCA analyses

data generated at different core labs. Moreover, only very few clinical trials have been published demonstrating data on the variability of the analyses performed, which in fact is a potential bias in the comparison of data among clinical trials.

## BASIC PRINCIPLES OF QCA

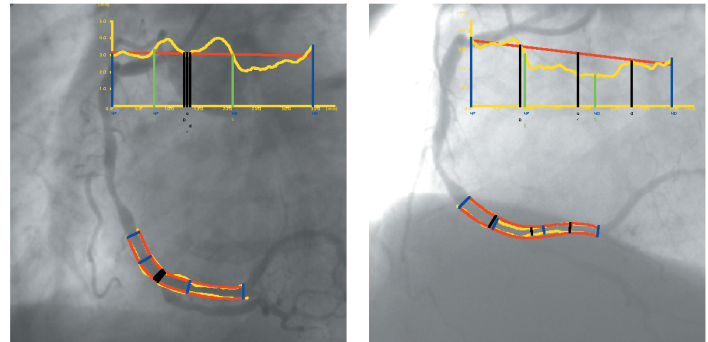
For a good understanding of variability in quantitative image analysis, it is important to know what the potential sources of variability for each imaging modality are. Although very important, image quality and signal-to-noise ratio are not the only factors involved. For example, the quality may be excellent, but if at two different points in time the calibration device and/or the artery of interest is not similarly visualised on an angiogram (see Figure 1), a proper comparison cannot be made. This will be explained below, using QCA as an example.

### Image Acquisition

The primary objective of a clinical trial is to quantify changes in time, comparing an angiogram prior to the start of the therapy with angiograms obtained during and/or at the end of the treatment. From an acquisition point of view, it is therefore critical that the same views are repeated, the same doses of intracoronary nitroglycerine doses are administered, the catheters (used for contrast injection) have minimal dimensions (preferably six French or higher, and no less than five), the same contrast media are applied, and whenever possible the same catheterisation room is used.

## Figure 1: Visualisation by Angiography

For comparison of quantitative analysis data from angiograms at different time points it is important that the same views are used. In this example there is a clear difference between the post-intervention angiogram (left) and the follow-up angiogram (right). For clinical interpretation this may not be a problem, whereas for quantitative analysis this may introduce significant variability in outcome data.

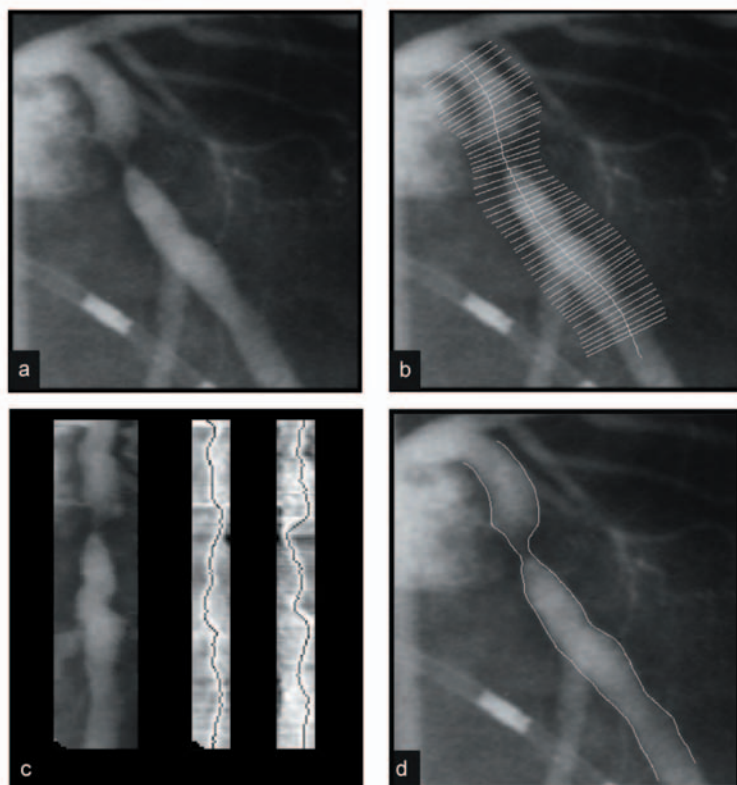


### QCA Analysis

With respect to the analysis software, the general characteristics and principles should be as follows. The operator initiates contour detection of the coronary artery segment of interest by defining the start and end-points of that segment, after which an arterial path line is automatically computed (1). Through a minimal cost analysis (MCA) contour detection algorithm, the left and right vessel contours are detected. If, on the judgement of the operator, manual adjustments must be made, a local MCA iteration is

**Figure 2: Basic Principles of Automated Contour Detection**

For the minimum cost analysis (MCA) contour-detection algorithm, the coronary segment of interest must first be selected (a); after which the scan lines are defined (b). Based on local changes in brightness level along the scan lines the optimal contour path along the entire segment is computed (c). Contours are then returned to the original image, after which the measurements are performed (d).



**Table 1: General Angiographic Views for QCA**

Left Coronary Artery	Right Coronary Artery
RAO 30°	LAO 30° to 45°
RAO 15° to 30° + 25° caudal	RAO 30° to 45°
RAO 0° to 30° + 40° cranial	Lateral 90°
LAO 45° to 55° with or without 25° cranial	AP + 20° cranial
Lateral 90°	

automatically re-applied, searching for the optimal final contours (see Figure 2).

For the quantitation of the image data, calibration of the image must first be performed. In coronary angiography, a non-tapering part of the contrast filled catheter is used. A similar MCA edge detection algorithm is applied, however including in the algorithm certain knowledge that the boundaries of the catheter are parallel. After calibration, the contours of the artery segment are defined from the proximal to the distal segment landmarks by an automated contour detection. Subsequently, the diameter function and reference diameter function are calculated by the software program, from which the quantitative outcome data (such as obstruction length, minimum lumen diameter, reference diameter, percentage diameter stenosis) are ultimately generated.

### SOURCES OF VARIABILITY

Keeping in mind the basic principles of QCA, validation of core lab data directly relates to the sources of

variability, which have an origin in the above-described elements at acquisition and offline independent QCA analysis at a core lab. The acquisition of angiograms in clinical trials is driven by the acquisition guidelines provided within the study protocol as well as by the experience and judgement of the investigator. Visualisation of coronary arteries must be done in such a way that the target segment of interest shows minimal foreshortening, no overlap with other vessels or catheters and the severity of the stenosis is maximally presented. General acquisition guidelines are listed in Table 1, but depending on the study these may have to be adapted or further specified.

For standardisation purposes, it is universally accepted that maximal coronary vasodilatation is a prerequisite. If vasodilatation is sub-optimal or even absent, the dimensions of the coronary artery can differ significantly between two time points; hence a bias in the quantitative data is introduced. It is, therefore, the task of the core lab to verify whether acquisition is done according to the guidelines provided and to give feedback to maintain or improve the quality of the image acquisition procedures when required.

Whichever analytical software program is used for QCA analysis, it is important that the software is well validated and fulfils the regulatory requirements on computerised systems validations. As this is an extensive topic itself and beyond the scope of this article, the accuracy of such a software program determines the precision of the quantitative data. Validation must demonstrate the true strengths, weaknesses and the clinical validity of a software package. For QCA, the accuracy of software can be compared with guidelines for variability (2) (see Table 2). Moreover, these guidelines can also be used as a reference for the (core

lab) observer variability in QCA analysis for clinical trials, which is an important factor in core lab validation. In addition to the quality of image acquisition, the QCA analysis process itself also determines the degree of observer variability, although this is reducing with new improvements in the QCA analysis techniques. The most important but also critical link in the analysis process is the calibration. Variable image quality of the catheters displayed and the size of the catheter have a large impact on all quantitative measurements. If the number of pixels is too

**Table 2: QCA Guidelines for Variability**

Study Type	Systematic Error (mm)	Random Error (mm)
Plexiglass phantom		
Off patient	<0.10	0.10-0.13
On patient	<0.10	0.10-0.13
<i>In vivo</i> study		0.10-0.20
Post mortem study		0.20-0.30
Inter/intra-observer variability		0.10-0.15
Short-term variability		0.15-0.25
Medium-term variability		0.20-0.30
Long-term variability		0.20-0.30

small (such as catheters smaller than five French), significant variation does occur. Thus, when only catheters of four French or smaller are available for calibration purposes, relative data (that is per cent diameter stenosis, and thus not absolute vessel dimensions) can be provided. Furthermore, when the catheter and the coronary artery segment are positioned at different distances from the image intensifier of the X-ray system, out-of-plane magnification occurs, affecting the reliability of the analysis results.

In clinical trials involving coronary angiography, QCA analysis is usually done for each coronary artery segment, from one major side branch to the next major side branch, as recommended by the American Heart Association and American College of Cardiology. The acquisition of angiograms in the catheterisation room must be done consistently when comparing angiographic data at different time points. Angiographic views that are not the same do result in different degrees of foreshortening (see Figure 1, see page 51). Furthermore, in the case of overlap of the target segment with, for example, another artery vessel or the catheter, manual corrections to the automated contour cannot be avoided. This adds variability to the data or, if manual correction cannot be done with sufficient accuracy, the image should even be rejected. However, variability does not only have its origins in acquisition. Standardisation of the QCA analysis process using validated software is also a pre-requisite (3). Based on the angiographic views available, only those frames that can meet certain criteria can be selected for subsequent analysis: good image contrast, appropriate image intensifier size, preferably end-diastolic frame (minimal cardiac motion), no overlap and minimal foreshortening. Furthermore, given the many tools that can be applied for different (complex) lesions and special therapies, the analysis procedures must be well described in standard operating procedures and the core lab analysis experts must have undergone thorough documented training to deal with all above difficulties. For core labs this can only be achieved if a validation programme is in place.

### CORE LAB ANALYSIS VALIDATION

With the increasing numbers and sizes of trials which are carried out in parallel in a core lab, it is of utmost importance to assess at a regular basis the inter- and intra-observer variability within the core lab, thereby making sure that each core lab technician is still meeting the strict requirements for offline core lab analysis. To minimise variability as much as possible, training and variability assessment programmes, standard operating procedures and detailed training on the large variety of real

world applications in clinical trials, are essential. Maintenance of training records and levels of experience is thus another vital element. For a trial sponsor or a regulatory body, the auditing of these documents is more or less standard routine. However, to monitor, audit and judge the validity of the quantitative data, requires a more in-depth knowledge and understanding of the inter- and intra-observer variability data and of specific parameters generated during the analysis process that can be used as an intrinsic quality control of the analysis process.

One method to assess the inter- and intra-observer variability, requires that all analysis experts at a core lab carry out the analysis of a fully blinded series of angiograms, randomised for each analysis expert and reflecting the variety of images to be obtained during a clinical trial. For this purpose, the sources of variability as described above are important and should contain images requiring manual interaction, the use of different software tools and different types of lesions and coronary artery segments. For the intra-observer variability, sufficient time (for example at least six weeks) should elapse between the sessions and the randomisation of the angiograms must be different. Finally, the statistical outcome of these variability assessments can be compared with the guidelines of variability for QCA (see Table 2, page 53). Such an approach does not just allow for intra-core lab variability, but also inter-core lab variability. An example of the outcome of such an assessment is summarised in Table 3, showing that in the case of proper standardisation, the systematic errors between two independent core labs, which use similar equipment and follow the exact same operating procedures, can be very small and that the random errors are within the ranges of variability from the reference values.

### CONCLUSION

For the validation of image analyses at core laboratories, it is crucial to have a solid understanding of the sources of variability in imaging. The key steps within the process of core lab validation can thus be defined and monitored, as shown for QCA analysis in clinical trials. Although the importance of core lab validation is well recognised, only limited data are available in literature, and variability data are only rarely included in articles describing the outcome of clinical trials. Not only from a scientific point of view, but also for regulatory reasons, the consistency and accuracy of data from clinical trials should be known, irrespective of whether these data are generated by quantitative image analyses, blinded readings or visual interpretations. This can only be achieved when such analysis variability data are provided together with the clinical trial data. ♦

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**Table 3: Core Laboratory Variability**

Inter-laboratory systematic and random errors in the individual measurements for two highly standardised core laboratories applying the same SOPs for QCA analysis.		
N = 63	Systematic Error (mm)	Random Error (mm)
Obstruction diameter (mm)	-0.06	0.14
Reference diameter (mm)	-0.02	0.15
Percentage Diameter Stenosis (%)	1.83	4.96
Mean Segment Diameter (mm)	0.00	0.10