

# Stability of Crick assay, and comparison with ROCHE assay

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08 July, 2020

## Introduction

The purpose of this analysis is to summarise:

- the stability of the Crick S1 protein ELISA assay
- the agreement between the Crick assay and the ROCHE assay

To achieve this, 45 samples that tested positive and 40 samples that tested negative using the Roche assay, were run through the Crick ELISA assay. Each sample was run in duplicate within each plate, and the assay was repeated a total of 5 times. Note that, as the Crick assay has a sample capacity of 40 samples per plate, the Roche-positive samples were split into batches of 40 and 5.

## Intra-assay variation

To summarise the intra-assay variation, the percentage coefficient of variation (% CV) is calculated for the duplicate readings of each sample, per plate. The mean % CV  $\pm$  standard deviation for the Roche-positive and Roche-negative samples are shown below.

Roché result	Intra-assay % CV	n
Positive	9.45 $\pm$ 7.12	225
Negative	8.53 $\pm$ 7.44	200

## Inter-assay variation

To summarise the inter-assay variation, the percentage coefficient of variation (% CV) is calculated for readings of each sample, across all its plates. The mean % CV  $\pm$  standard deviation for the Roche-positive and Roche-negative samples are shown below.

Roché result	Intra-assay % CV	n
Positive	12.93 $\pm$ 7.6	45
Negative	16.4 $\pm$ 5.78	40

## Inter-assay outcome stability and agreement with Roche assay

In addition to summarizing the variability of absorbance measurements, it's also important to summarize the variability in well outcome. The Crick ELISA assay has three possible outcomes per well:

- detected
- not detected
- indeterminate

while the Roche assay has only detected and not detected. The table below shows the frequencies of each outcome across each plate replicate of the first 40 Roche-positive samples.

Plate_replicate	Detected	Indeterminate	Not detected
A	70	6	4
B	73	4	3
C	71	5	4
D	68	8	4
E	68	8	4

The information in the table above is summarised below as a matrix of Jaccard distances. The Jaccard distance indicates the percentage of disagreement between the well outcomes of two plates.

```
##           1           2           3           4
## 2 0.07228916
## 3 0.02469136 0.04878049
## 4 0.04878049 0.11764706 0.07228916
## 5 0.04878049 0.11764706 0.07228916 0.00000000
```

The same table and matrix are shown below but for the additional 5 Roche-positive samples.

Plate_replicate	Detected	Indeterminate	Not detected
A	8	2	0
B	8	2	0
C	3	3	4
D	6	2	2
E	6	2	2

```
##           1           2           3           4
## 2 0.00000000
## 3 0.66666667 0.66666667
## 4 0.33333333 0.33333333 0.4615385
## 5 0.33333333 0.33333333 0.4615385 0.00000000
```

Below, the percentage of wells of Roche-positive samples that are also Crick-positive, is summarised (84.67%).

Detected	Indeterminate	Not detected	Percentage agreement with Roche
381	42	27	84.67

The same is performed below for the Roche-negative samples. Note that all of these samples had an outcome of not detected by the Crick assay.

Plate_replicate	Not detected
A	80
B	80
C	80
D	80
E	80

The Jaccard distances are therefore all 0.

```
## 1 2 3 4
## 2 0
## 3 0 0
## 4 0 0 0
## 5 0 0 0 0
```

The agreement with the Roche assay is 100%.

Not detected	Percentage agreement with Roche
400	100

## Accuracy, specificity and sensitivity

The confusion matrix below shows the Crick assay outcomes (rows) against the Roche assay outcomes (columns). As the Crick assay has three possible outcomes but the Roche assay has only two, indeterminate samples from the Crick assay are classified as not detected for the calculations below.

	Detected	Not detected
Detected	381	0
Not detected	69	400

The overall accuracy across all wells is 91.88%, considering the Roche result as a ground truth. Additional summary statistics for this confusion matrix are shown below where:

- sensitivity is the proportion of Roche-positive samples that were also Crick-positive
- specificity is the proportion of Roche-negative samples that were also Crick-negative
- Precision is the proportion of Crick-positive samples that were also Roche-positive
- F1 is the harmonic mean of sensitivity and precision

```
## Sensitivity Specificity Precision F1
## 0.8466667 1.0000000 1.0000000 0.9169675
```