# WWARN In Vitro Data Management and Analysis Report

Sample Report

28-Feb-2013

# Contents


**1 Introduction** **2**

**2 Methods** **3**

2.1 Percentage conversion . . . . . 3

2.2 Non-linear regression . . . . . 3

2.3 Core Criteria for summary analyses . . . . . 3

2.4 Range warnings . . . . . 3

**3 Results Summary** **5**

3.1 Data Analysis Summary . . . . . 5

**4 Summary** **6**

4.1 Field Summary . . . . . 6

4.1.1 Assay Summary . . . . . 6

4.1.2 Field isolates summary statistics . . . . . 6

4.1.3 Annual $IC_{50}$ plots by drug . . . . . 6

4.2 3D7 Summary . . . . . 9

4.2.1 Assay Summary . . . . . 9

4.2.2 3D7 isolates summary statistics . . . . . 9

**A ANNEX** **10**

A.1 Field Samples . . . . . 10

A.2 3D7 Samples . . . . . 44

# 1 Introduction

A key aim of the Worldwide Antimalarial Resistance Network (WWARN) is to monitor geospatial and temporal trends in antimalarial drug resistance. In vitro drug susceptibility remains a central pillar of antimalarial efficacy surveillance. The assessment of antimalarial drug susceptibility in parasites straight from patients (ex vivo parasites that are not culture-adapted) is largely independent of clinical factors and hence provides information that complements clinical assessment of drug efficacy. The WWARN In Vitro Module aims to enhance the amount of in vitro data available, increase scientific capacity in endemic countries and provide intelligence on resistance or susceptibility to various antimalarials.

Determination of the in vitro susceptibility of field P. falciparum isolates to antimalarial drugs is undertaken via a variety of measurement systems and analytical approaches. These, as well as variation in culture methods, have developed according to specific research questions, characteristics of the study site or simple logistical constraints. Such variations in design, methodology, analysis and presentation of in vitro studies constitute a challenge for the collation of data from different centres. WWARN's approach to this issue, across all modules, is to work with complete sets of primary (raw) data, allowing the characteristics of the methodology to be understood, and analyses to be undertaken via a standardised approach. For in vitro studies, the primary data are the raw output from ex vivo assessment of drug effects on an individual isolate for a single drug. In almost all cases, the dataset for an isolate comprises many such individual assessments for each of several different drugs.

WWARN notes that individual researchers may take different approaches to data management and analysis. It is inevitable that WWARN-derived drug susceptibility results will vary to some extent compared to analyses performed by the data contributor. Any differences do not reflect a value judgment as to which analytical approach is correct. A uniform approach is taken only to apply standardised methodologies and minimise bias on geospatial and temporal trends derived from the many studies compiled in the Data Repository.

# 2 Methods

Submitted data are extracted and transformed into a standardised format, ensuring that all 'no drug' controls on an individual plate are applied to the $IC_{50}$ calculation for each drug. The transformation method will vary from study to study and will be developed by the module curator along with the WWARN Informatics team. All data handling steps will be documented.

## 2.1 Percentage conversion

Experimental data are converted to a percentage scale based on:

- 100% value: $E(C_0)$ defined as the mean effect across all wells on a plate which contain no drug.
- 0% value: $E_{min}$ defined as the average effect over the two concentrations with the lowest mean effect for a particular drug.

## 2.2 Non-linear regression

A sigmoid, 4-parameter concentration-inhibition model is applied to the data based on established approaches (Le Nagard et al 2010), with the upper constraint set at 100% and the lower at 0% i.e. $E(C_0)$ and $E_{min}$. This final step yields two key parameters for each curve, the $IC_{50}$ and gamma (an expression of the slope of the curve at the $IC_{50}$), each with 95% confidence intervals.

## 2.3 Core Criteria for summary analyses

Confidence in the $IC_{50}$ value and slope resulting from linear regression will be used to define a subset of results with a tight confidence interval suitable for core analyses and reports according to the following core criteria:

- If gamma is not 10, ratio of upper : lower 95% confidence intervals for $IC_{50}$ must be less than 3 (and both confidence intervals positive)
- If gamma is 10 (indicating a fixed, steep slope), ratio of $E(C_0)$ : $E_{min}$ must be greater than 2 indicating acceptable growth (Basco 2007)

Note: On some occasions, non-linear regression fails to converge or produce an acceptable curve, due to either a very steep slope or noisy data. Under such circumstances, the non-linear regression algorithm repeats the process with a fixed gamma of 10. If this second iteration is successful, this renders the process a 1-parameter model. Such curves are consequently heavily constrained (at top, bottom and slope) so that the confidence intervals for $IC_{50}$ (the last variable parameter) may be insensitive measures of true confidence. For this reason the additional criterion based on signal and noise is applied in such cases.

## 2.4 Range warnings

With some datasets there is evidence that the range of drugs used to determine $IC_{50}$ is too high or too low, and Range warnings are accordingly triggered. Range warnings are applied independently of core criteria so assays that meet core criteria, but trigger a range warning, are retained in core analyses.

**The 'Range high' warning is triggered when $IC_{50}$ < lowest drug concentration.** The drug range is so high that even the lowest drug concentration already inhibits growth by more than 50%.

**The 'Range low' warning is triggered when $(E(C_0) - EC_{max}) > (1.05 \times (E(C_0) - E_{min}))$.** This is designed to pick up 'unfinished assays' where there is evidence that further inhibition would have occurred if a higher range of concentrations had been used. $E(C_0) - EC_{max}$ is the efficacy of the drug measured at the highest drug concentration while $E(C_0) - E_{min}$ is the normal measure of drug efficacy used by IVART. In assays where $E(C_0) - EC_{max}$ is more than $E(C_0) - E_{min}$ efficacy is continuing to increase at the highest concentration; a threshold of 1.05 corresponds to an additional increase in inhibition of 10% at Cmax compared to any other concentration.

# 3 Results Summary

All data analysed by the analysis and reporting tool are presented below. Fitted assays indicate the number of assays where the data points could be successfully fitted to a curve using non-linear regression. Core assays indicate the number of assays that meet the core criteria (presented in 2.3).

## 3.1 Data Analysis Summary

| Sample type | Samples | Drugs | Sites | Assays | Fitted Assays | Core Assays |
|---|---|---|---|---|---|---|
| Field | 34 | 9 | 1 | 263 | 244 | 210 |
| 3D7 | 4 | 8 | 1 | 16 | 16 | 16 |

# 4 Summary

## 4.1 Field Summary

### 4.1.1 Assay Summary

The number of assays, fitted assays and core assays (defined in 2.3) per drug are presented below. Range refers to a warning for assays where an inadequate range of drug concentrations was used to test the isolate (defined in 2.4). The range can be either too high or too low.

| Drug | Assays | Fitted Assays | Core Assays | Range |
|---|---|---|---|---|
| AV | 2 | 2 | 2 | 0 |
| CQ | 33 | 32 | 29 | 1 |
| DHA | 33 | 30 | 27 | 1 |
| DOX | 32 | 30 | 25 | 0 |
| DQ | 33 | 29 | 27 | 0 |
| LUM | 32 | 29 | 24 | 0 |
| MQ | 33 | 30 | 25 | 1 |
| PIP | 33 | 31 | 25 | 0 |
| QN | 32 | 31 | 26 | 0 |

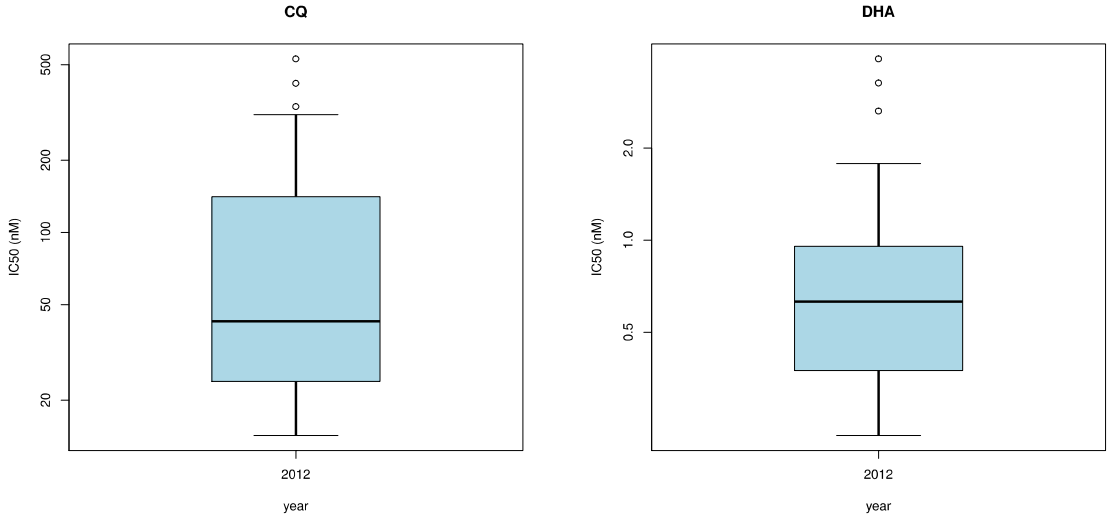
### 4.1.2 Field isolates summary statistics

All results where at least 10 assays meet the core criteria per drug per year are summarized in the table below.

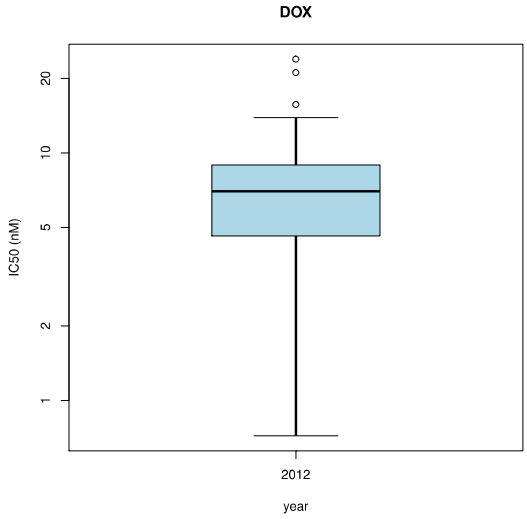
| Year | Drug | Number of isolates | Geometric mean of $IC_{50}$ |
|---|---|---|---|
| 2012 | CQ | 29 | 60.426 |
| 2012 | DHA | 27 | 0.678 |
| 2012 | DOX | 25 | 5.511 |
| 2012 | DQ | 27 | 17.669 |
| 2012 | LUM | 24 | 5.167 |
| 2012 | MQ | 25 | 13.591 |
| 2012 | PIP | 25 | 16.677 |
| 2012 | QN | 26 | 108.655 |

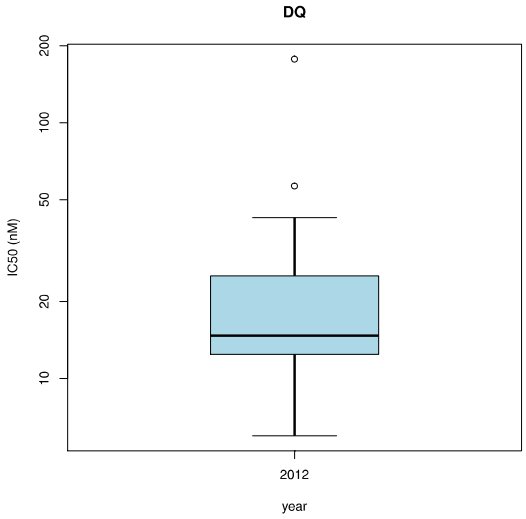
### 4.1.3 Annual $IC_{50}$ plots by drug

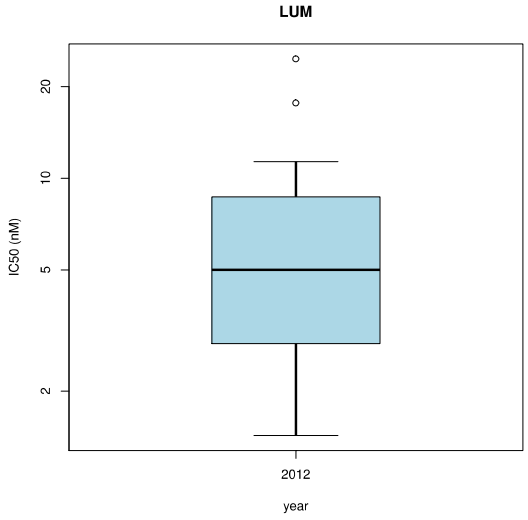
Box plots show median, quartiles and range for $IC_{50}$ values for each drug and year.

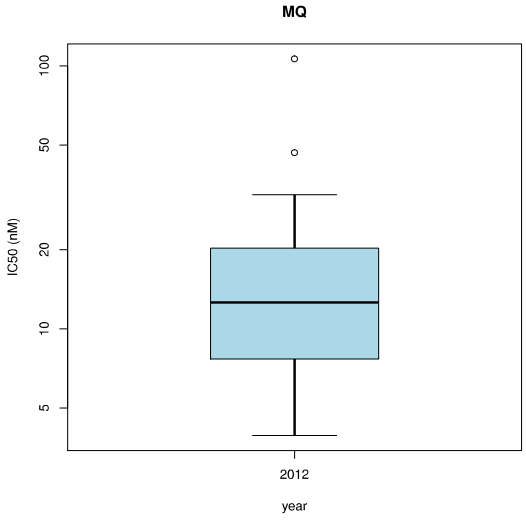

CQ
IC50 (nM)
500
200
100
50
20
2012
year

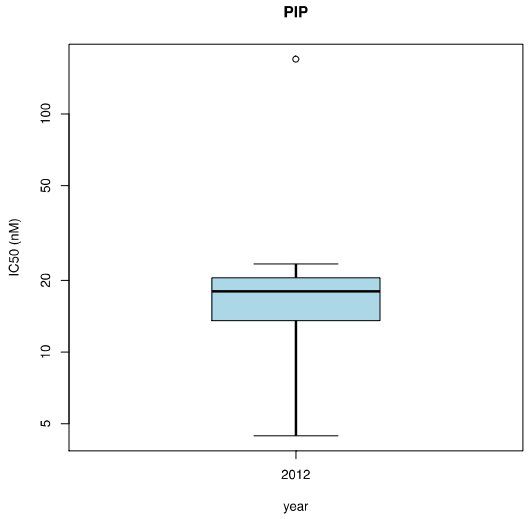

DHA
IC50 (nM)
2.0
1.0
0.5
2012
year

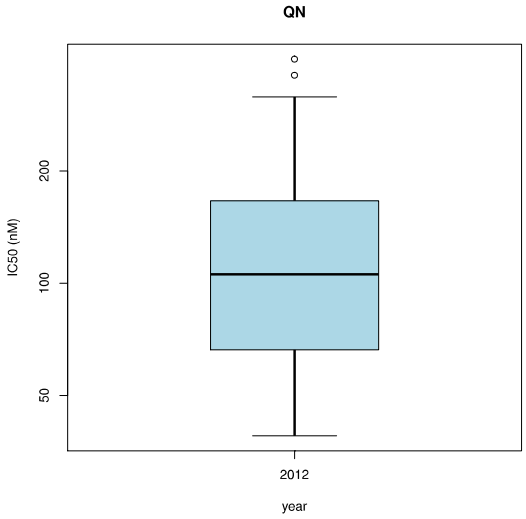

DOX
IC50 (nM)
20
10
5
2
1
2012
year


DQ
IC50 (nM)
200
100
50
20
10
2012
year


LUM
IC50 (nM)
2
5
10
20
2012
year


MQ
IC50 (nM)
5
10
20
50
100
2012
year


PIP
IC50 (nM)
5
10
20
50
100
2012
year


QN
IC50 (nM)
50
100
200
2012
year

## 4.2 3D7 Summary

### 4.2.1 Assay Summary

The number of assays, fitted assays and core assays (defined in 2.3) per drug are presented below. Range refers to a warning for assays where an inadequate range of drug concentrations was used to test the isolate (defined in 2.4). The range can be either too high or too low.

| Drug | Assays | Fitted Assays | Core Assays | Range |
|---|---|---|---|---|
| CQ | 2 | 2 | 2 | 0 |
| DHA | 2 | 2 | 2 | 0 |
| DOX | 2 | 2 | 2 | 0 |
| DQ | 2 | 2 | 2 | 0 |
| LUM | 2 | 2 | 2 | 0 |
| MQ | 2 | 2 | 2 | 0 |
| PIP | 2 | 2 | 2 | 0 |
| QN | 2 | 2 | 2 | 0 |

### 4.2.2 3D7 isolates summary statistics

All assays meeting the core criteria per drug per year are summarized in the table below.

| Year | Drug | Number of isolates | Geometric mean of $IC_{50}$ |
|---|---|---|---|
| 2012 | CQ | 2 | 33.305 |
| 2012 | DHA | 2 | 1.665 |
| 2012 | DOX | 2 | 10.42 |
| 2012 | DQ | 2 | 11.784 |
| 2012 | LUM | 2 | 20.591 |
| 2012 | MQ | 2 | 39.188 |
| 2012 | PIP | 2 | 22.383 |
| 2012 | QN | 2 | 130.711 |

# A ANNEX

## A.1 Field Samples

Individual results for each isolate are presented below with the following outputs from analysis: $IC_{50}$, lower and upper 95% confidence intervals of $IC_{50}$ and their ratio and gamma. Additional columns indicate whether the assay passed core criteria (defined in 2.3) and if there is concern over the range of drug concentrations used (defined in 2.4).

### Sample 2012ANG0011-712345028-1-22/8/2012

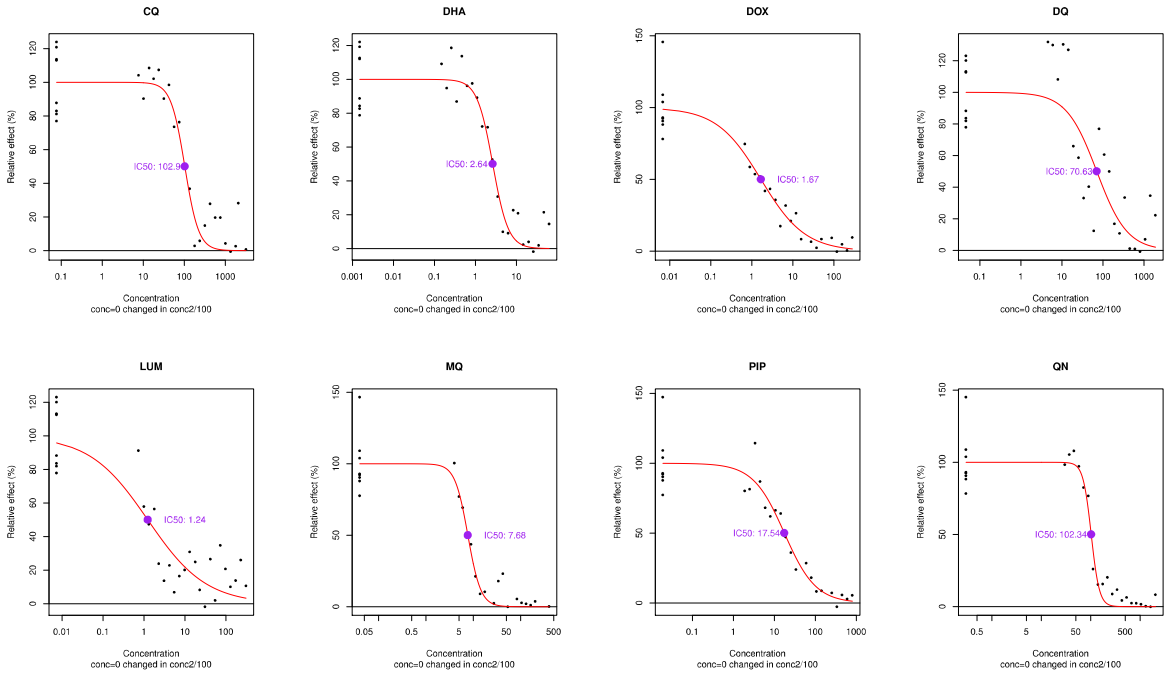
Date: 22/8/2012

Country: GA

Assays: 8

| Drug | $IC_{50}$ (95% CI) | Lower CI | Upper CI | Ratio | Gamma | Meets Core Criteria |
|---|---|---|---|---|---|---|
| CQ | 102.9 | 78.07 | 127.73 | 1.64 | 2.56 | yes |
| DHA | 2.64 | 2.03 | 3.24 | 1.59 | 2.29 | yes |
| DOX | 1.67 | 0.93 | 2.4 | 2.59 | 0.81 | yes |
| DQ | 70.63 | 27.72 | 113.55 | 4.1 | 1.17 | no |
| LUM | 1.24 | 0.1 | 2.38 | 24.84 | 0.61 | no |
| MQ | 7.68 | 6.34 | 9.01 | 1.42 | 3.18 | yes |
| PIP | 17.54 | 11.24 | 23.84 | 2.12 | 1.13 | yes |
| QN | 102.34 | 89.01 | 115.66 | 1.3 | 5.25 | yes |

### Plots for sample 2012ANG0011-712345028-1-22/8/2012



## Sample 2012AVC0010-712305010-1-23/7/2012

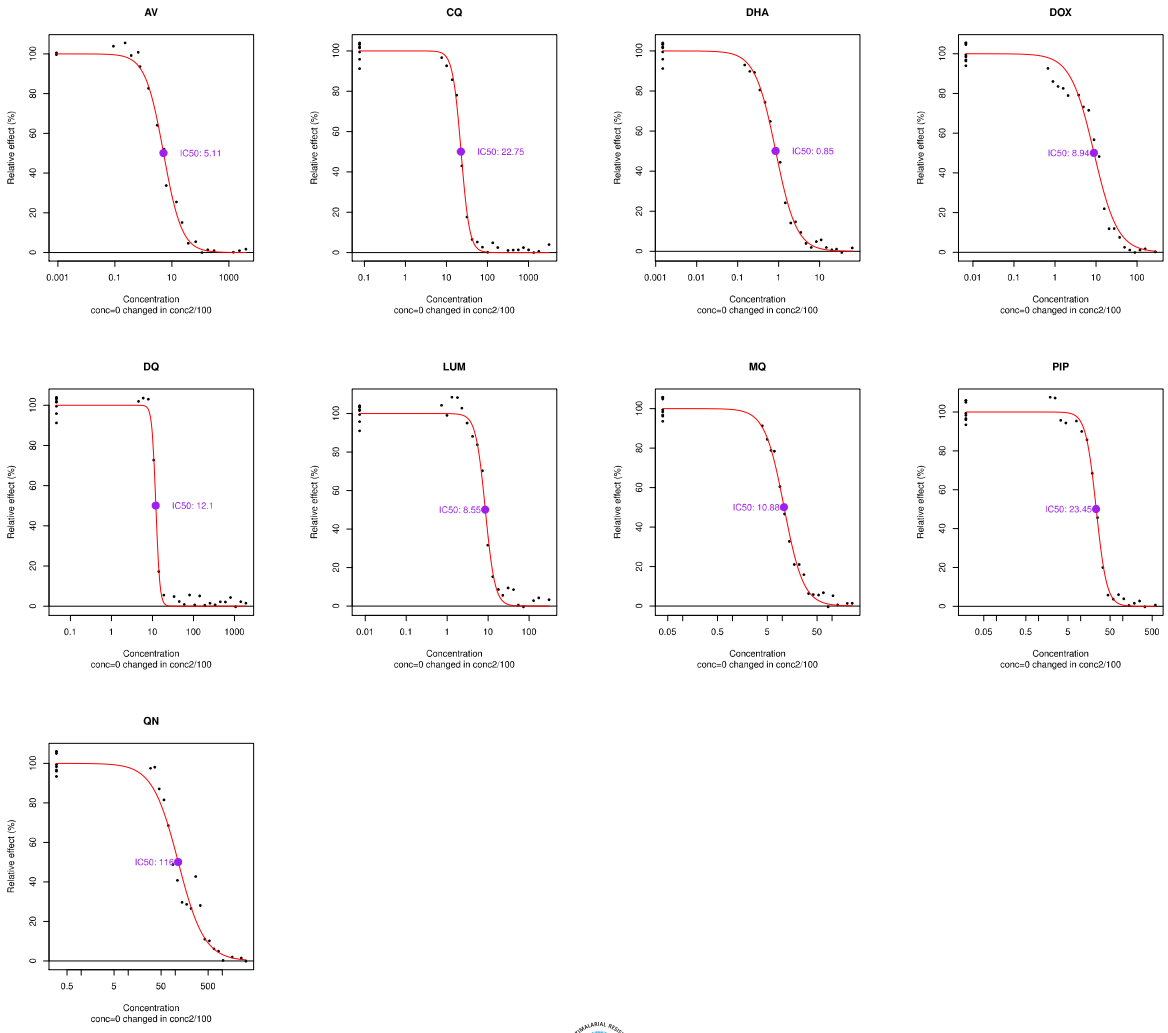
Date: 23/7/2012

Country: TG

Assays: 9

| Drug | $IC_{50}$ (95% CI) | Lower CI | Upper CI | Ratio | Gamma | Meets Core Criteria |
|---|---|---|---|---|---|---|
| AV | 5.11 | 4.49 | 5.72 | 1.28 | 1.35 | yes |
| CQ | 22.75 | 21.8 | 23.7 | 1.09 | 4.22 | yes |
| DHA | 0.85 | 0.79 | 0.9 | 1.14 | 1.71 | yes |
| DOX | 8.94 | 7.61 | 10.27 | 1.35 | 1.51 | yes |
| DQ | 12.1 | 11.7 | 12.49 | 1.07 | 9.08 | yes |
| LUM | 8.55 | 7.96 | 9.13 | 1.15 | 3.71 | yes |
| MQ | 10.88 | 10.26 | 11.51 | 1.12 | 2.24 | yes |
| PIP | 23.45 | 22.07 | 24.83 | 1.12 | 3.48 | yes |
| QN | 116 | 98.76 | 133.23 | 1.35 | 1.57 | yes |

## Plots for sample 2012AVC0010-712305010-1-23/7/2012



## Sample 2012AVC0014-712335019-1-14/8/2012

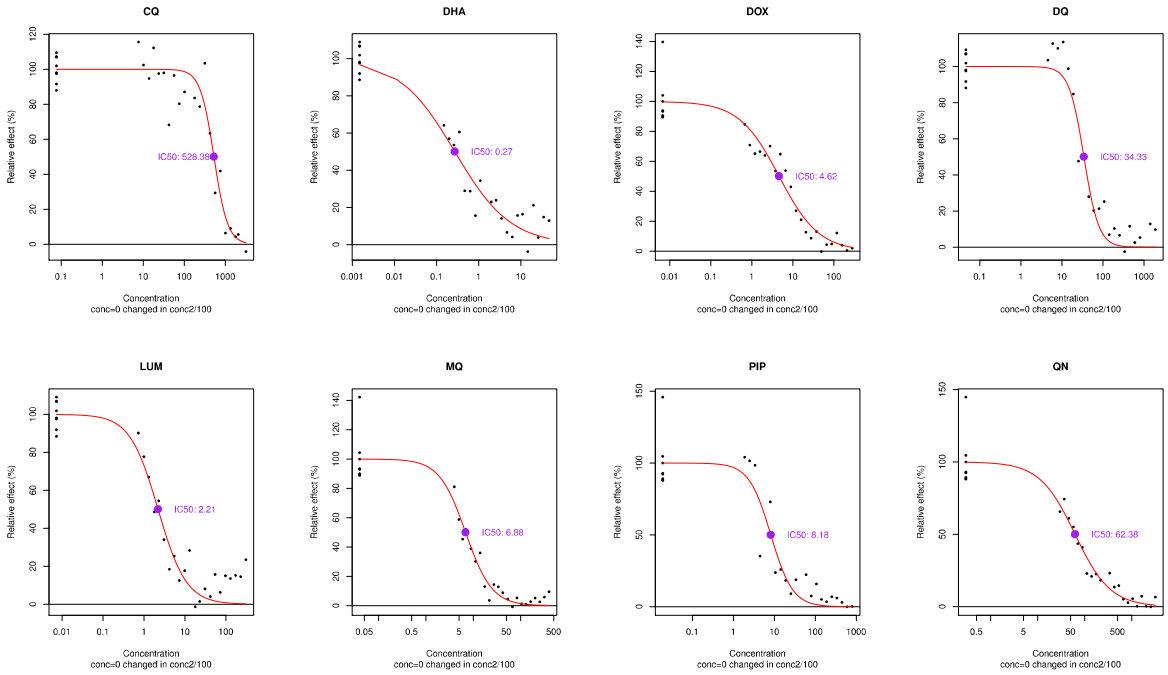
Date: 14/8/2012

Country: ML

Assays: 8

| Drug | $IC_{50}$ (95% CI) | Lower CI | Upper CI | Ratio | Gamma | Meets Core Criteria |
|---|---|---|---|---|---|---|
| CQ | 528.38 | 423.91 | 632.85 | 1.49 | 2.6 | yes |
| DHA | 0.27 | 0.15 | 0.39 | 2.61 | 0.65 | yes |
| DOX | 4.62 | 3.17 | 6.07 | 1.91 | 0.89 | yes |
| DQ | 34.33 | 27.23 | 41.44 | 1.52 | 2.39 | yes |
| LUM | 2.21 | 1.64 | 2.77 | 1.69 | 1.26 | yes |
| MQ | 6.88 | 5.35 | 8.4 | 1.57 | 1.57 | yes |
| PIP | 8.18 | 5.21 | 11.16 | 2.14 | 1.63 | yes |
| QN | 62.38 | 44.8 | 79.96 | 1.78 | 1.15 | yes |

## Plots for sample 2012AVC0014-712335019-1-14/8/2012



## Sample 2012CCH0019-712345018-1-21/8/2012

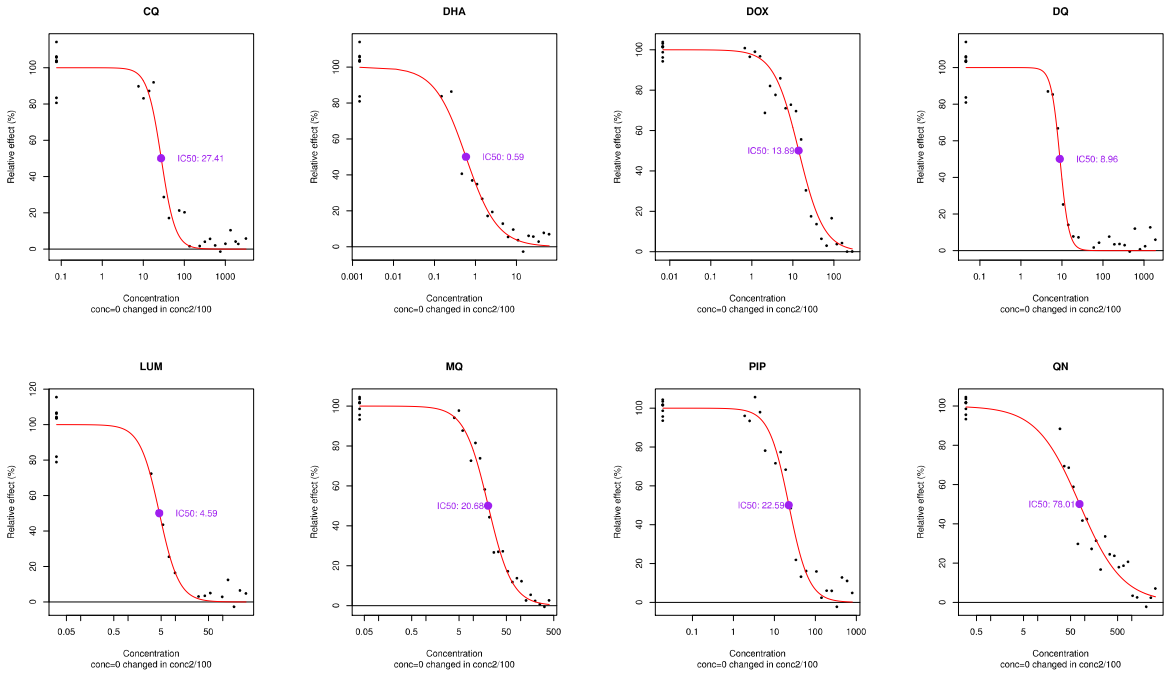
Date: 21/8/2012

Country: TG

Assays: 8

| Drug | $IC_{50}$ (95% CI) | Lower CI | Upper CI | Ratio | Gamma | Meets Core Criteria |
|---|---|---|---|---|---|---|
| CQ | 27.41 | 22.03 | 32.78 | 1.49 | 2.55 | yes |
| DHA | 0.59 | 0.44 | 0.73 | 1.66 | 1.13 | yes |
| DOX | 13.89 | 11.49 | 16.28 | 1.42 | 1.42 | yes |
| DQ | 8.96 | 8 | 9.92 | 1.24 | 4.04 | yes |
| LUM | 4.59 | 3.73 | 5.46 | 1.47 | 2.11 | yes |
| MQ | 20.68 | 18.92 | 22.45 | 1.19 | 1.72 | yes |
| PIP | 22.59 | 19.26 | 25.93 | 1.35 | 1.78 | yes |
| QN | 78.01 | 60.6 | 95.42 | 1.57 | 0.95 | yes |

## Plots for sample 2012CCH0019-712345018-1-21/8/2012



## Sample 2012CCH0020-712345032-1-22/8/2012

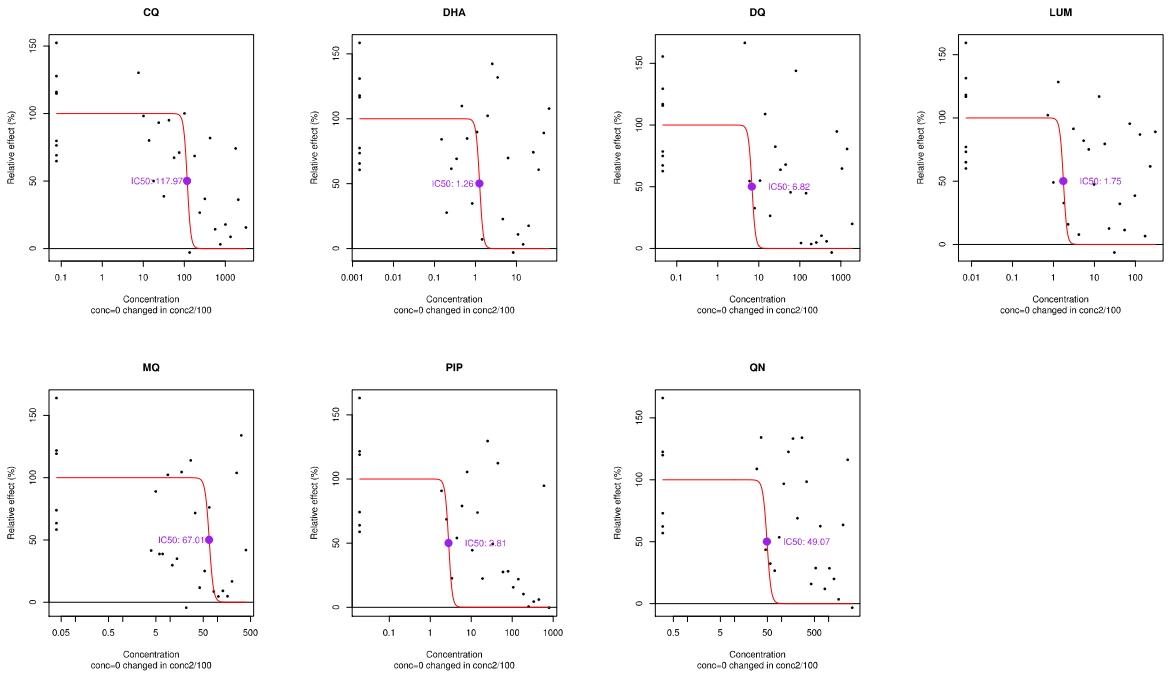
Date: 22/8/2012

Country: CM

Assays: 7

| Drug | $IC_{50}$ (95% CI) | Lower CI | Upper CI | Ratio | Gamma | Meets Core Criteria |
|---|---|---|---|---|---|---|
| CQ | 117.97 | 78.08 | 157.86 | 2.02 | 10 | no |
| DHA | 1.26 | 0.56 | 1.97 | 3.54 | 10 | no |
| DQ | 6.82 | 3.32 | 10.32 | 3.1 | 10 | no |
| LUM | 1.75 | 0.99 | 2.51 | 2.55 | 10 | no |
| MQ | 67.01 | 39.58 | 94.43 | 2.39 | 10 | no |
| PIP | 2.81 | 1.34 | 4.28 | 3.19 | 10 | no |
| QN | 49.07 | 24.75 | 73.38 | 2.96 | 10 | no |

## Plots for sample 2012CCH0020-712345032-1-22/8/2012



## Sample 2012TNN0018-712335018-1-14/8/2012

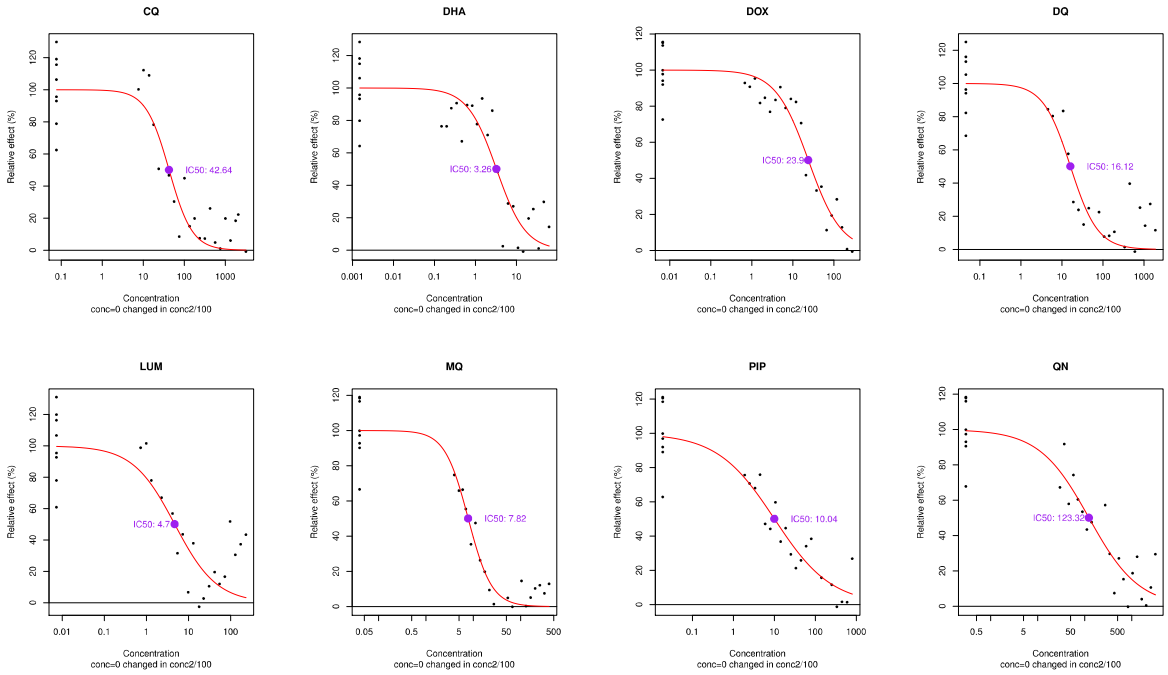
Date: 14/8/2012

Country: CI

Assays: 8

| Drug | $IC_{50}$ (95% CI) | Lower CI | Upper CI | Ratio | Gamma | Meets Core Criteria |
|---|---|---|---|---|---|---|
| CQ | 42.64 | 25.27 | 60.02 | 2.38 | 1.54 | yes |
| DHA | 3.26 | 1.82 | 4.69 | 2.57 | 1.23 | yes |
| DOX | 23.9 | 16.75 | 31.06 | 1.85 | 1.08 | yes |
| DQ | 16.12 | 9.08 | 23.17 | 2.55 | 1.31 | yes |
| LUM | 4.7 | 1.19 | 8.22 | 6.93 | 0.87 | no |
| MQ | 7.82 | 6.12 | 9.52 | 1.55 | 1.75 | yes |
| PIP | 10.04 | 4.79 | 15.29 | 3.19 | 0.62 | no |
| QN | 123.32 | 70.07 | 176.58 | 2.52 | 0.82 | yes |

## Plots for sample 2012TNN0018-712335018-1-14/8/2012



### Sample 2012bcb0062-712340253-1-23/8/2012

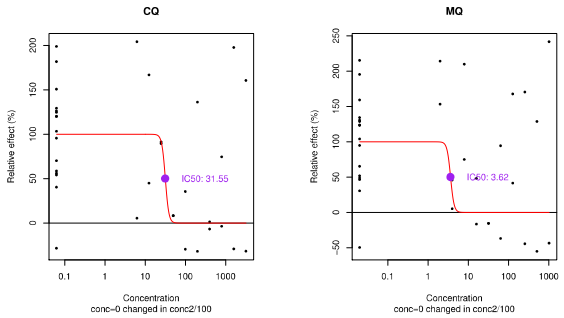
Date: 23/8/2012

Country: CM

Assays: 4

| Drug | $IC_{50}$ (95% CI) | Lower CI | Upper CI | Ratio | Gamma | Meets Core Criteria |
|---|---|---|---|---|---|---|
| CQ | 31.55 | -7.16 | 70.27 | -9.81 | 10 | no |
| DHA | No fit | | | | | |
| DQ | No fit | | | | | |
| MQ | 3.62 | 1.22 | 6.01 | 4.91 | 10 | no |

### Plots for sample 2012bcb0062-712340253-1-23/8/2012



## A.2 3D7 Samples

Individual results for each isolate are presented below with the following outputs from analysis: $IC_{50}$, lower and upper 95% confidence intervals of $IC_{50}$ and their ratio and gamma. Additional columns indicate whether the assay passed core criteria (defined in 2.3) and if there is concern over the range of drug concentrations used (defined in 2.4).

### Sample 3D7-10/7/2012-1-10/7/2012

Date: 10/7/2012

Country:

Assays: 4

| Drug | $IC_{50}$ (95% CI) | Lower CI | Upper CI | Ratio | Gamma | Meets Core Criteria |
|---|---|---|---|---|---|---|
| CQ | 32.5 | 30.49 | 34.51 | 1.13 | 4.37 | yes |
| DHA | 1.66 | 1.5 | 1.81 | 1.21 | 1.74 | yes |
| DQ | 11.93 | 11.49 | 12.37 | 1.08 | 10 | yes |
| LUM | 21.72 | 18.67 | 24.77 | 1.33 | 1.54 | yes |

### Plots for sample 3D7-10/7/2012-1-10/7/2012