

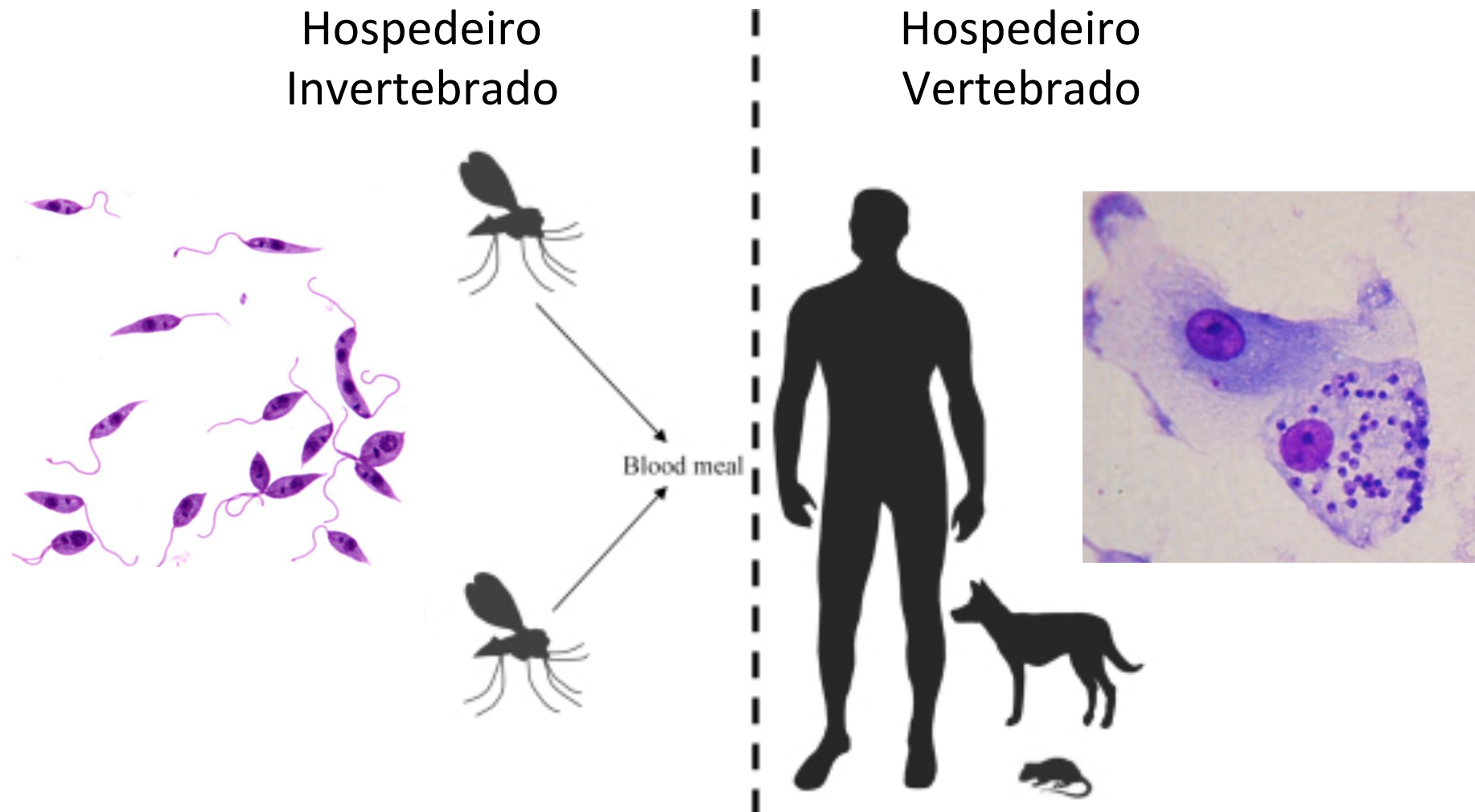
# Avaliação da sensibilidade de *Leishmania* a fármacos

Caroline Ricce Espada

caroline.respada@gmail.com

Aula prática 13/06/2017

# Onde vive o parasita *Leishmania*?

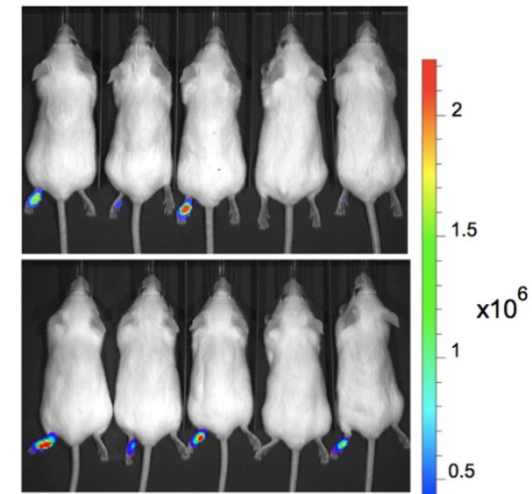
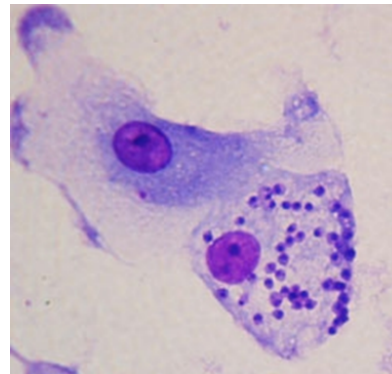


# Teste de fármacos em *Leishmania*

Promastigotas



Amastigotas



Qual modelo vocês escolheriam para testar seu fármaco?

Qual a relevância do teste em promastigotas?

# Como avaliar sensibilidade das promastigotas?

## Viabilidade celular

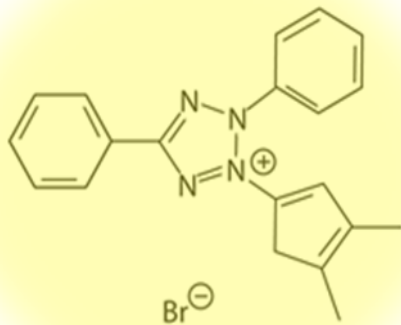
- **Aplicações**
  - Citotoxicidade (perda de células viáveis)
  - Diferentes tipos celulares (eucariotas)
    - Promastigotas de *Leishmania* spp.
    - Epimastigotas de *Trypanosoma cruzi*
    - Macrófagos e linhagens tumorais, dentre outras
- **Ensaio colorimétrico**
  - Sensível, quantitativo, reprodutível
  - Baixo custo

# Ensaio de MTT

- **Princípio**

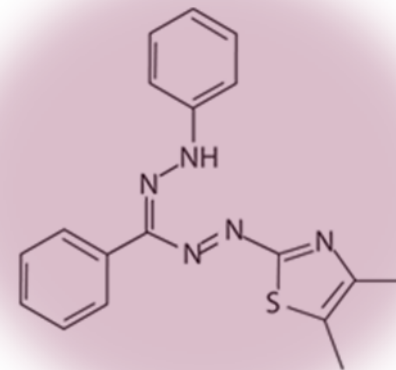
- **MTT** (3-[4,5-dimetiltiazol-2il]-2,5-difeniltetrazolium)

- Sal solúvel **amarelo**
    - Reduzido a **formazan** (**púrpura**) por desidrogenases mitocondriais



MTT

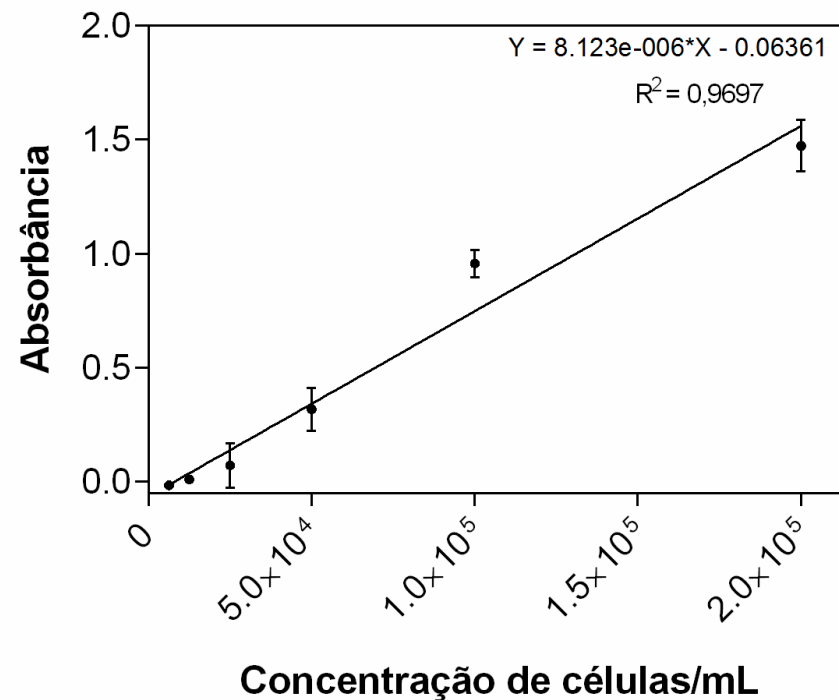
Desidrogenases  
mitocondriais



Formazan

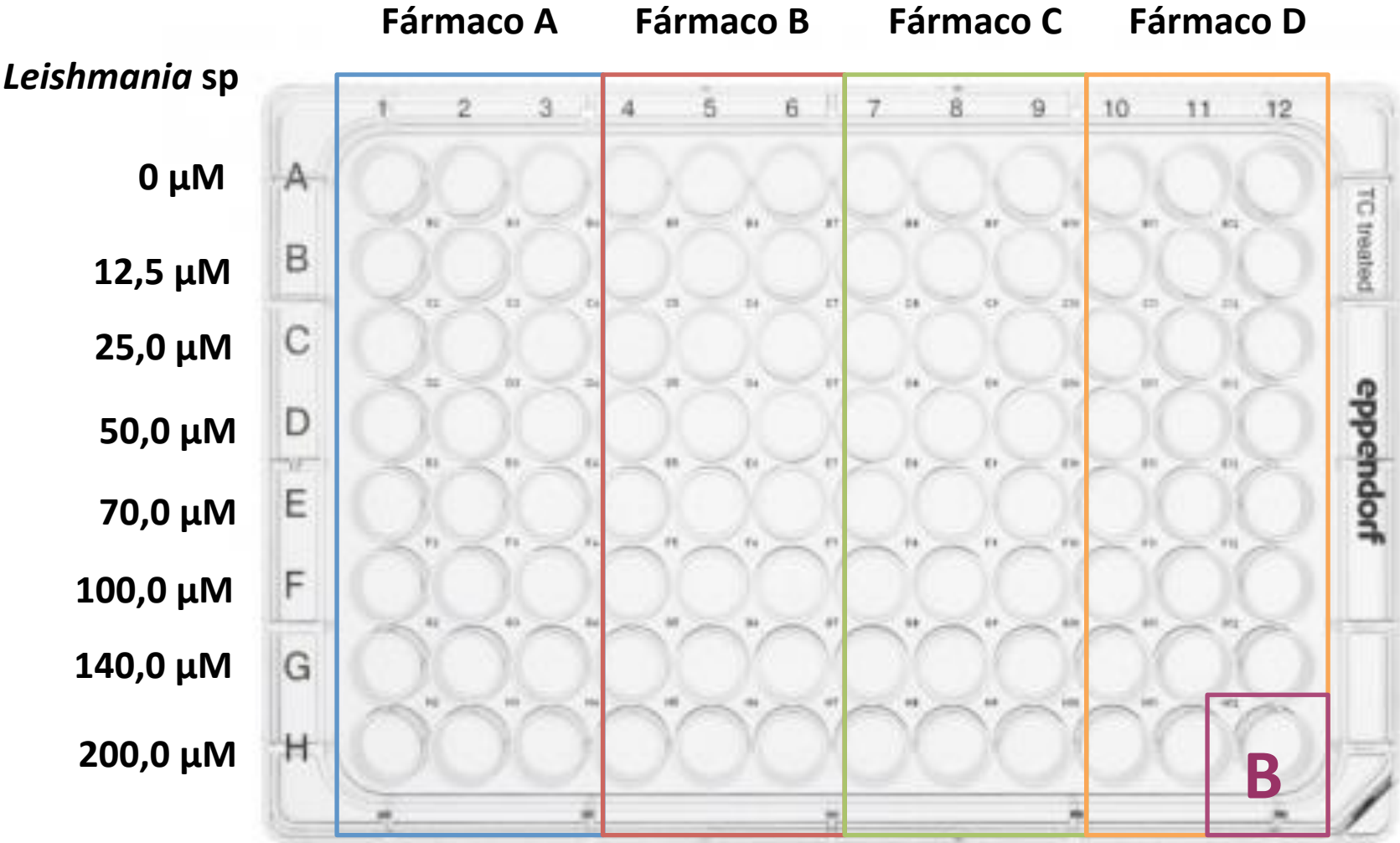
# Ensaio de MTT

- A quantidade de formazan produzida é diretamente proporcional ao número de células viáveis na amostra.



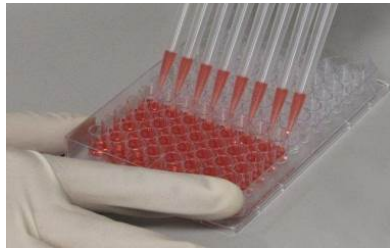
		Leish A			Leish B			Leish C			Leish D		
Fármaco X		1	2	3	4	5	6	7	8	9	10	11	12
0 $\mu$ M	A												
12,5 $\mu$ M	B												
25,0 $\mu$ M	C												
50,0 $\mu$ M	D												
70,0 $\mu$ M	E												
100,0 $\mu$ M	F												
140,0 $\mu$ M	G												
200,0 $\mu$ M	H												

B

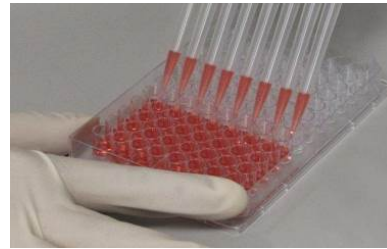
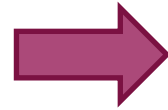




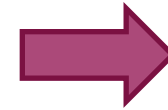
# Procedimento



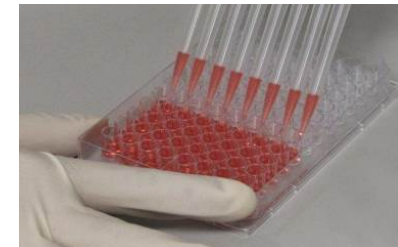
Plaqueamento  
de fármacos em diferentes  
concentrações



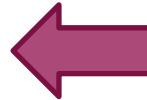
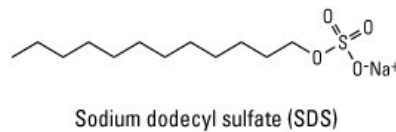
Plaqueamento  
*L. amazonensis*  
(promastigotas)  
Cultivo em meio M-199



Incubação  
24 h à 25 °C



Adição de MTT  
Incubação por 4 horas

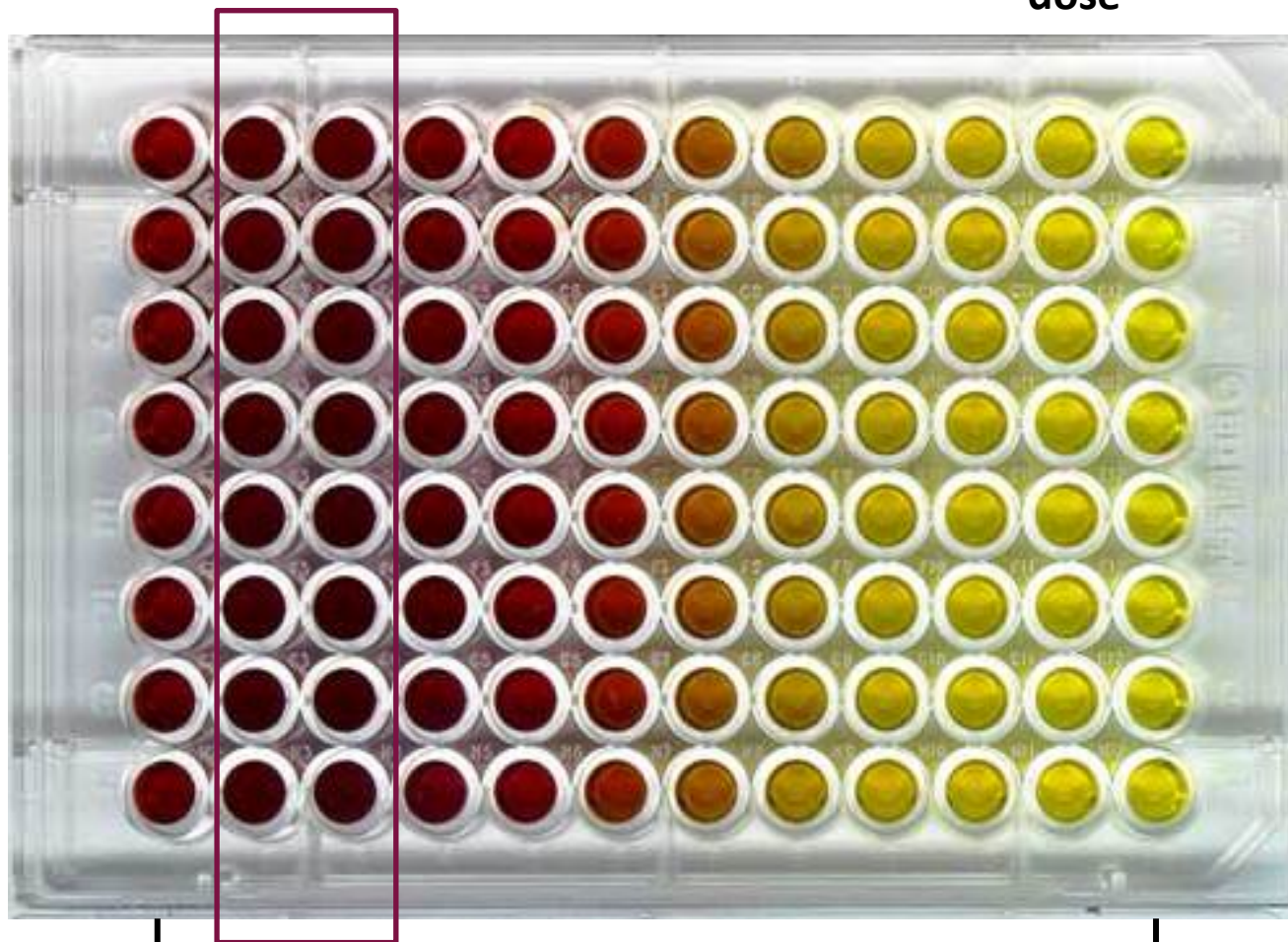


Medição da absorbância  
595 nm e 690 nm

# Resultado

0  $\mu$ M

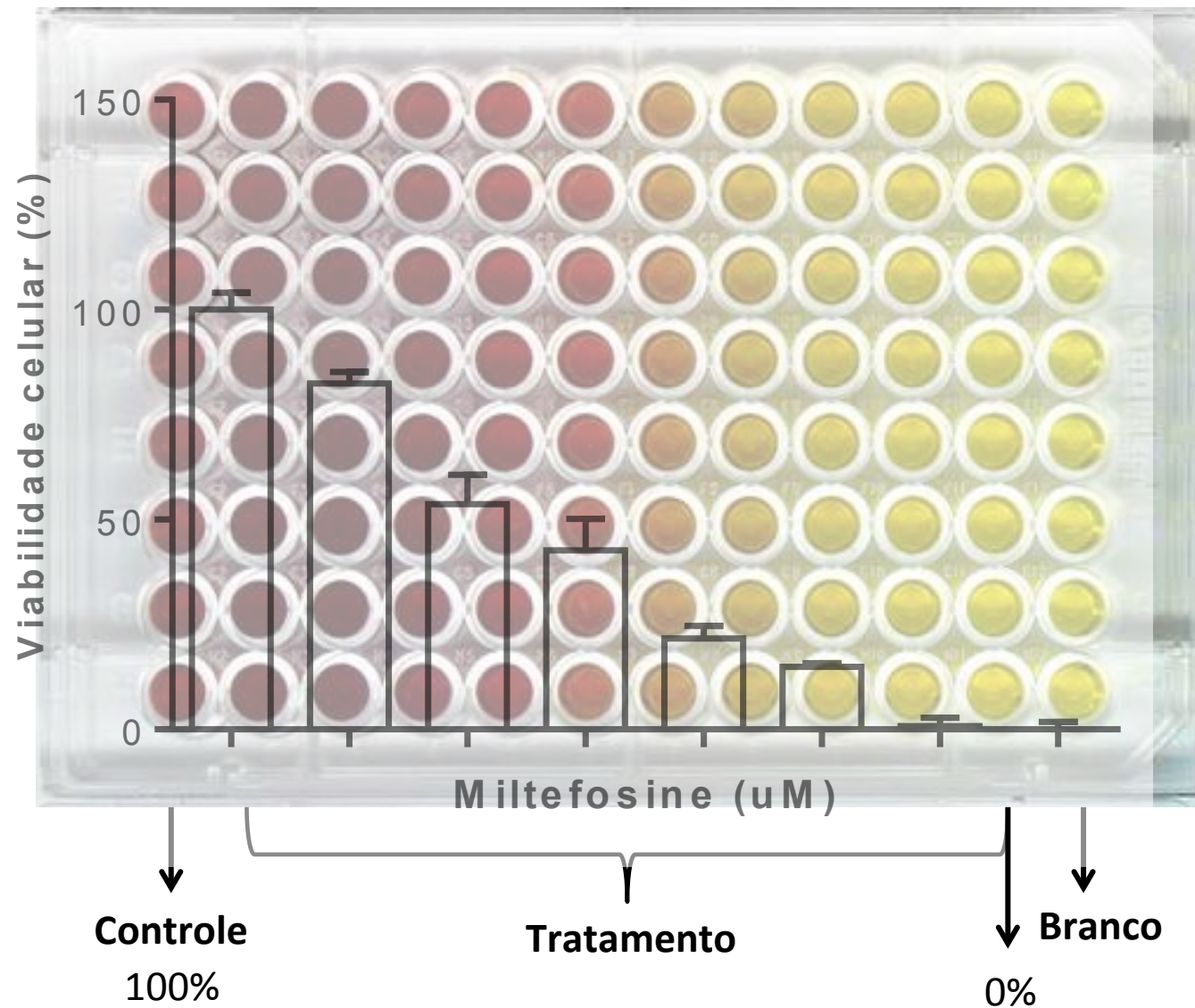
Maior  
dose



Células vivas

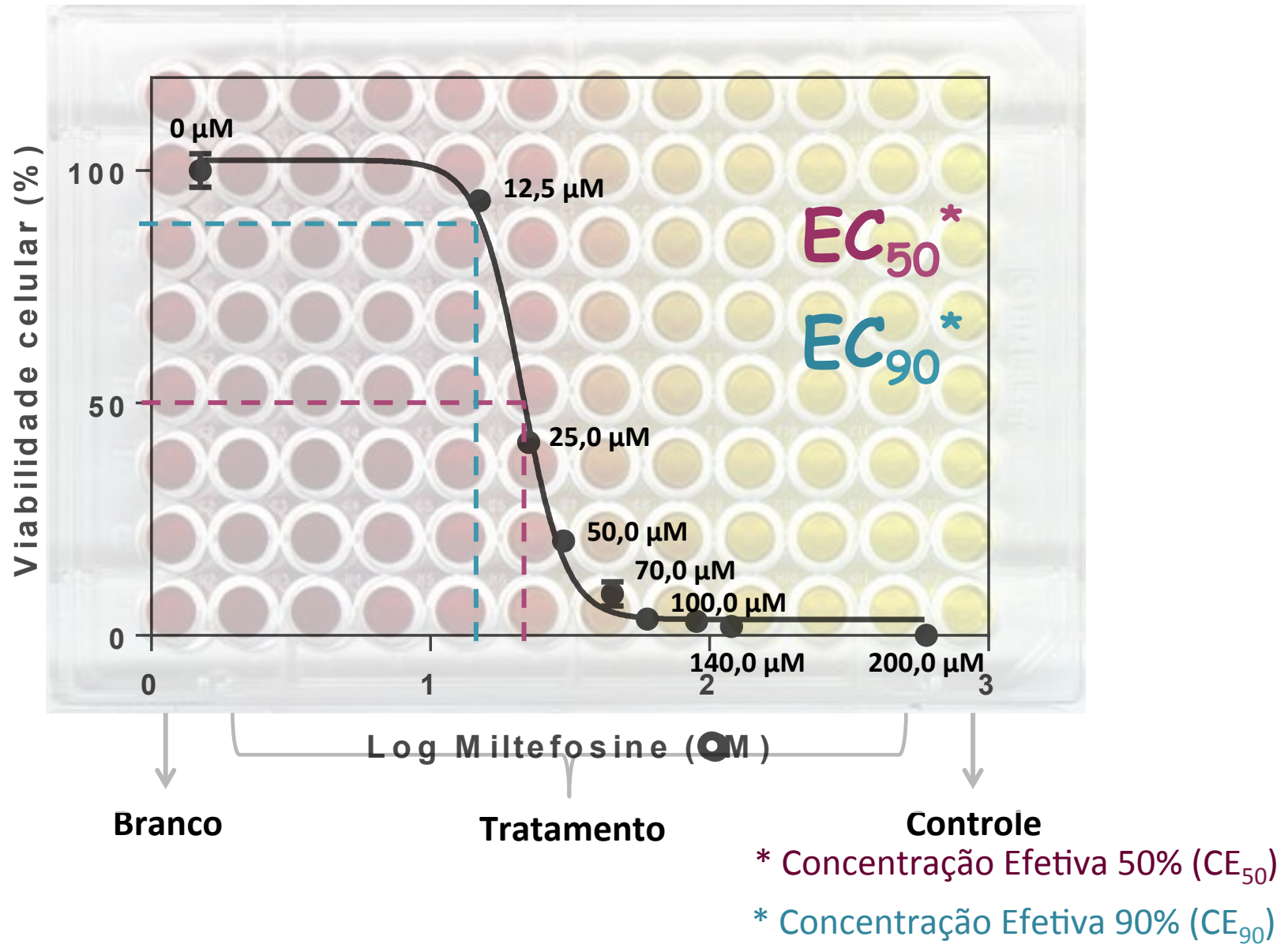
Células mortas

# Resultado





# Resultado




# Atividade prática

- Cada grupo (5 alunos) receberá uma folha contendo as DOs referentes a dois ensaios de MTT com isolados de *L. braziliensis* tratados com miltefosina e/ou anfotericina B
- Cada grupo deverá:
  - Construir um gráfico de histograma (média e desvio padrão) mostrando a % de viabilidade em cada concentração de fármaco.
  - Construir as curvas de  $CE_{50}$  e  $CE_{90}$  no programa Graph Prism 7.0.
  - Calcular o valor de  $CE_{50}$  e  $CE_{90}$  dos seus experimentos.
  - Comparar os resultados.

# Download Prisma 7.0

- Versão Trial (30 dias):

<https://www.graphpad.com/demos>



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Sign In

### Prism 7 Trial Registration

I'm ready to start my free 30 day trial subscription! Please email me the instructions to install and activate GraphPad Prism.

Email \*

Enter your email address.

Confirm Email \*


Confirm your email address.

First Name \*

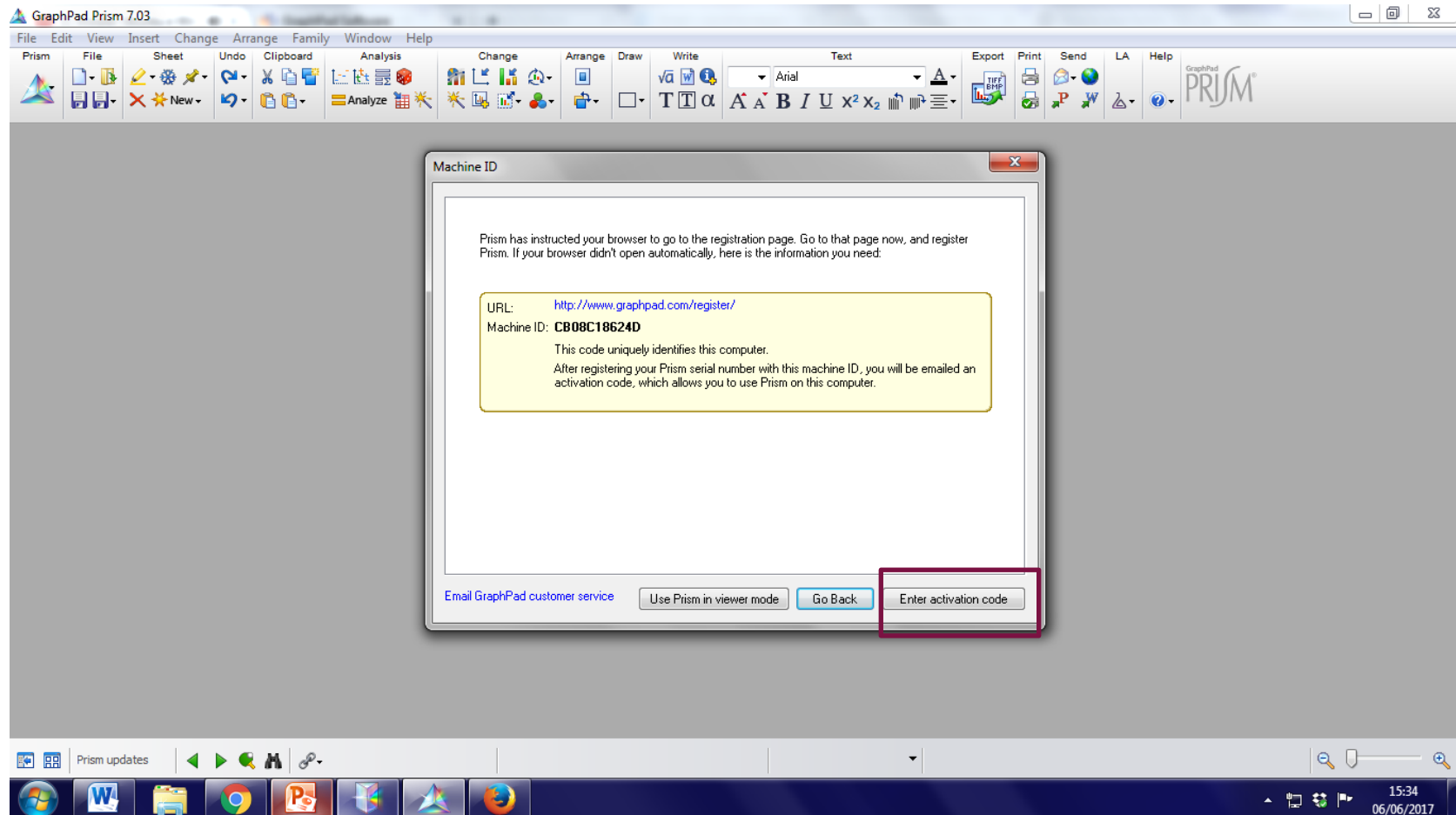
Last Name \*

Submit

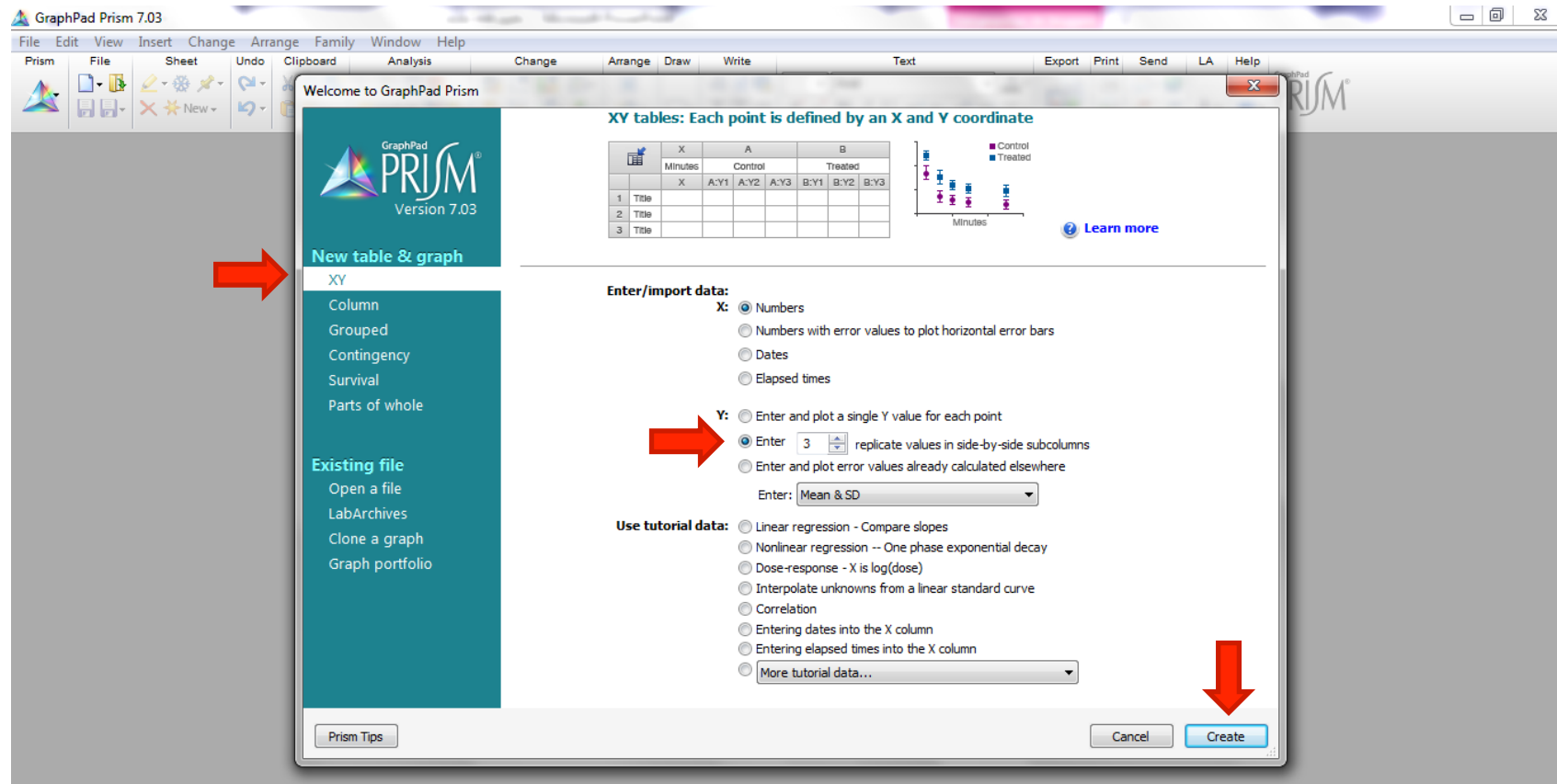
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# Download Prisma 7.0



# Construção de Curva de CE<sub>50</sub>





# Construção de Curva de CE<sub>50</sub>

GraphPad Prism 7.03 - [Project1:Data 1]

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Change Import Draw Write Text Export Print Send LA Help

Table format: XY

	X	Group A			Group B			Group C			Group D			Group E	
	Doses Miltefosina (µM)	Leitura MTT (D.O.)			Title			Title			Title			Title	
		A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3	C:Y1	C:Y2	C:Y3	D:Y1	D:Y2	D:Y3	E:Y1	E:Y2
1	0.01	1.107	1.075000	1.155000											
2	1.00	1.025	0.969000	1.028000											
3	3.00	1.029	0.926000	0.960000											
4	6.30	0.973	0.948000	0.916000											
5	13.00	0.784	0.819000	0.876000											
6	25.00	0.340	0.415000	0.387000											
7	50.00	0.043	0.056000	0.049000											
8	100.00	0.024	0.026267	0.026667											
9															
10															
11															
12															
13															
14															
15															
16															
17															
18															
19															
20															
21															
22															
23															
24															
25															

Family Search results Data Tables Data 1 Info Project info 1 Results Graphs Data 1 Layouts

Row 1, Column RT

# Construção de Curva de CE<sub>50</sub>

GraphPad Prism 7.03 - [Project1:Data 1]

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Change Import Draw Write Text Export Print Send LA Help

Family Search results Data Tables Data 1 Info Project info 1 Results Graphs Data 1 Layouts

Table format: XY X

1 0.01

2 1.60

3 3.00

4 6.30

5 13.00

6 25.00

7 50.00

8 100.00

9 Title

10 Title

11 Title

12 Title

13 Title

14 Title

15 Title

16 Title

17 Title

18 Title

19 Title

20 Title

21 Title

22 Title

23 Title

24 Title

25 Title

Group D Title

C:Y3 D:Y1 D:Y2 D:Y3 E:Y1 E:Y2

Group E Title

Which analysis?

Built-in analysis

Transform, Normalize...

Transform

Transform Concentrations (X)

Normalize

Prune rows

Remove baseline and column math

Transpose X and Y

Fraction of total

XY analyses

Nonlinear regression (curve fit)

Linear regression

Fit spline/LOWESS

Smooth, differentiate or integrate curve

Area under curve

Deming (Model II) linear regression

Column statistics

Row means with SD or SEM

Correlation

Interpolate a standard curve

Column analyses

Grouped analyses

Contingency table analyses

Analyze which data sets?

☒ A:Leitura MTT (D.O.)

When you analyze tables or graphs with more than one data set, use this space to select which data set(s) to analyze.

Select All Deselect All

Help Cancel OK

# Construção de Curva de CE<sub>50</sub>

GraphPad Prism 7.03 - [Project1:Data 1]

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Change Import Draw Write Text Export Print Send LA Help

Family Search results Data Tables Data 1 Info Project info 1 Results Graphs Data 1 Layouts

Table format: XY		X	Group A			Group B			Group C			Group D			Group E	
		Doses Miltefosina (µM)	Leitura MTT (D.O.)			Title			Title			Title			Title	
	X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3	C:Y1	C:Y2	C:Y3	D:Y1	D:Y2	D:Y3	E:Y1	E:Y2	
1		0.01	1.107	1.075000												
2	Title	1.60	1.025	0.969000												
3	Title	3.00	1.029	0.926000												
4	Title	6.30	0.973	0.948000												
5	Title	13.00	0.784	0.819000												
6	Title	25.00	0.340	0.415000												
7	Title	50.00	0.043	0.056000												
8	Title	100.00	0.024	0.026267												
9	Title															
10	Title															
11	Title															
12	Title															
13	Title															
14	Title															
15	Title															
16	Title															
17	Title															
18	Title															
19	Title															
20	Title															
21	Title															
22	Title															
23	Title															
24	Title															
25	Title															

Parameters: Row means with SD or SEM

Scope of calculations

- ☐ Calculate one total/mean for entire data table.
- ☒ Calculate a total/mean for each data set.

Calculate

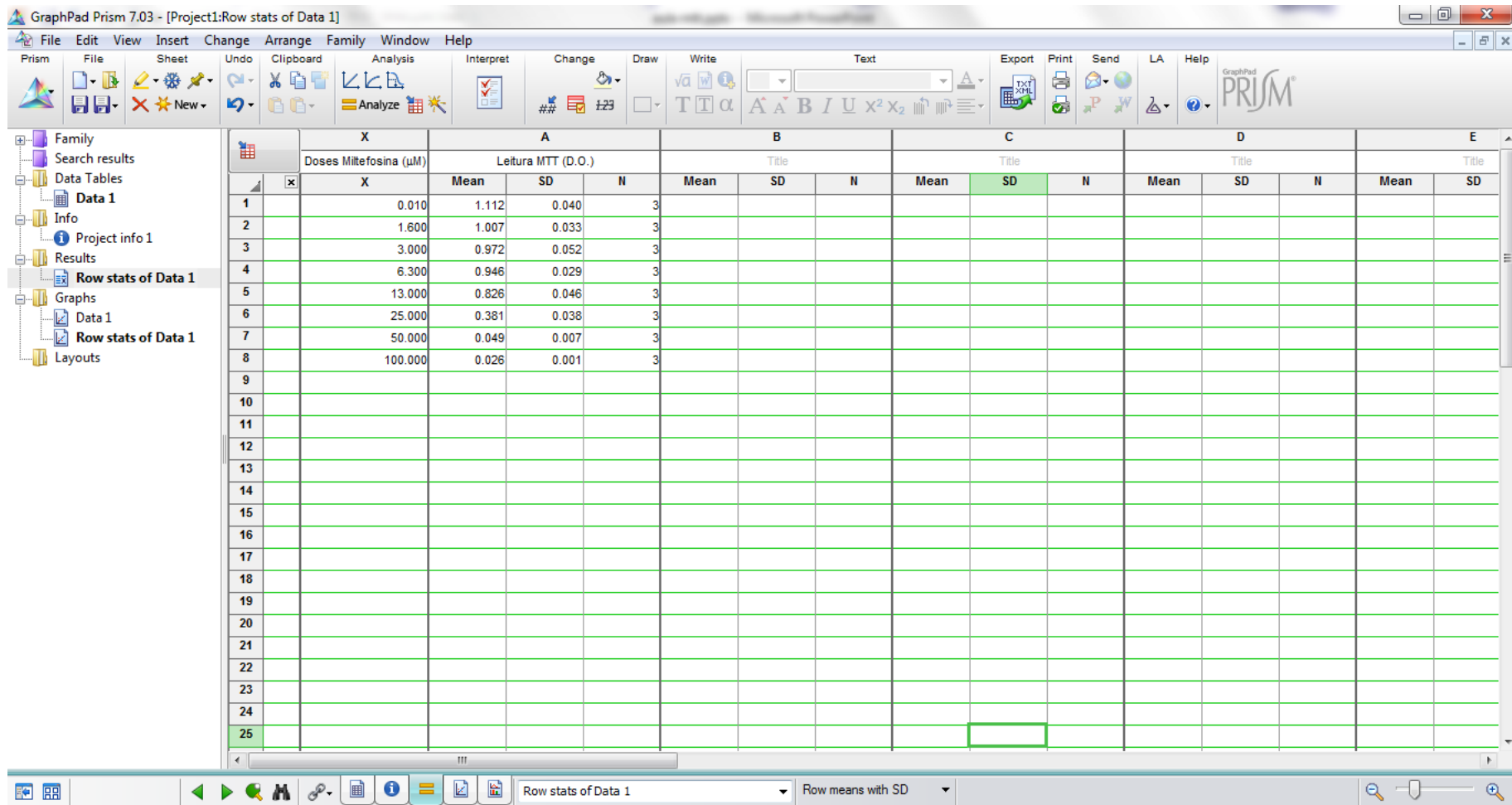
- ☐ Row totals
- ☐ Row means
- ☒ Row means with SD
- ☐ Row means with SEM
- ☐ Row means with %CV
- ☐ Row medians with range

New graph

- ☐ Create a new graph of the results

Learn Cancel OK

# Construção de Curva de CE<sub>50</sub>



# Construção de Curva de $CE_{50}$

GraphPad Prism 7.03 - [Project1:Row stats of Data 1]

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

Family  
Search results  
Data Tables  
Data 1  
Info  
Project info 1  
Results  
Row stats of Data 1  
Graphs  
Data 1  
Row stats of Data 1  
Layouts

Doses Miltefosina ( $\mu$ M)

	X
1	0.010
2	1.600
3	3.000
4	6.300
5	13.000
6	25.000
7	50.000
8	100.000
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	

Analyze Data

Built-in analysis

Which analysis?

- Transform, Normalize...
  - Transform
  - Transform Concentrations (X)
  - Normalize
  - Prune rows
  - Remove baseline and column math
  - Transpose X and Y
  - Fraction of total
- XY analyses
  - Nonlinear regression (curve fit)
  - Linear regression
  - Fit spline/LOWESS
  - Smooth, differentiate or integrate curve
  - Area under curve
  - Deming (Model II) linear regression
  - Column statistics
  - Row means with SD or SEM
  - Correlation
  - Interpolate a standard curve
- Column analyses
- Grouped analyses
- Contingency table analyses

Analyze which data sets?

☒ A:Leitura MTT (D.O.)

When you analyze tables or graphs with more than one data set, use this space to select which data set(s) to analyze.

Select All Deselect All

Help Cancel OK

Row stats of Data 1

Row means with SD

# Construção de Curva de $CE_{50}$

GraphPad Prism 7.03 - [Project1:Row stats of Data 1]

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

Family  
Search results  
Data Tables  
Data 1  
Info  
Project info 1  
Results  
Row stats of Data 1  
Graphs  
Data 1  
Row stats of Data 1  
Layouts

	X	A			
	Doses Miltefosina (µM)	Leitura MTT (D.O.)			
	X	Mean	SD	N	
1	0.010	1.112	0.040	3	
2	1.600	1.007	0.033	3	
3	3.000	0.972	0.052	3	
4	6.300	0.946	0.029	3	
5	13.000	0.826	0.046	3	
6	25.000	0.381	0.038	3	
7	50.000	0.049	0.007	3	
8	100.000	0.026	0.001	3	
9					
10					
11					
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24					
25					

Parameters: Normalize

**Subcolumns**

- ☒ Average the subcolumns, and normalize the means
- ☐ Normalize each subcolumn separately

**How is 0% defined?**

- ☒ Smallest value in each data set
- ☐ First value in each data set (or last, whichever is smaller)
- ☐ Remove from the results page
- ☐ Y=  becomes 0% for all data sets

**How is 100% defined?**

- ☒ Largest value in each data set
- ☐ Last value in each data set (or first, whichever is larger)
- ☐ Remove from the results page
- ☐ Y=  becomes 100% for all data sets
- ☐ The sum of all values in the data set (column)

**Present results as**

- ☐ Fractions
- ☒ Percentages

**New graph**

- ☒ Create a new graph of the results

Learn Cancel OK

# Construção de Curva de CE<sub>50</sub>

GraphPad Prism 7.03 - [Project1:Normalize of Row stats of Data 1]

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

Family  
Search results  
Data with Results  
Data 1  
...Row stats of Data 1  
.....Normalize of Row stats of Data 1  
Data Tables  
Info  
Project info 1  
Results  
Graphs  
Data 1  
Row stats of Data 1  
Normalize of Row stats of Data 1  
Layouts  
Floating Notes

	X
	Doses Miltefosina (uM)
1	0.010
2	1.600
3	3.000
4	6.300
5	13.000
6	25.000
7	50.000
8	100.000
9	
10	
11	
12	
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19	
20	
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22	
23	
24	
25	

Analyze Data

Built-in analysis

Which analysis?

- ☒ Transform, Normalize...
  - Transform
  - Transform Concentrations (X)
  - Normalize
  - Prune rows
  - Remove baseline and column math
  - Transpose X and Y
  - Fraction of total
- ☐ XY analyses
  - Nonlinear regression (curve fit)
  - Linear regression
  - Fit spline/LO/WESS
  - Smooth, differentiate or integrate curve
  - Area under curve
  - Deming (Model II) linear regression
  - Column statistics
  - Row means with SD or SEM
  - Correlation
  - Interpolate a standard curve
- ☐ Column analyses
- ☐ Grouped analyses
- ☐ Contingency table analyses

Analyze which data sets?

☒ A:Leitura MTT (D.O.)

When you analyze tables or graphs with more than one data set, use this space to select which data set(s) to analyze.

Select All Deselect All

Help Cancel OK

Normalize of Row stats of Data 1 Normalized data

# Construção de Curva de $CE_{50}$

GraphPad Prism 7.03 - [Project1:Normalize of Row stats of Data 1]

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

Family  
Search results  
Data with Results  
Data 1  
...Row stats of Data 1  
.....Normalize of Row stats of Data 1  
Data Tables  
Info  
Project info 1  
Results  
Graphs  
Data 1  
Row stats of Data 1  
Normalize of Row stats of Data 1  
Layouts  
Floating Notes

	X	
	Doses Miltefosina (µM)	
	X	M
1	0.010	
2	1.600	
3	3.000	
4	6.300	
5	13.000	
6	25.000	
7	50.000	
8	100.000	
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25		

Parameters: Transform

**Function List**

- ☒ Standard functions
- ☐ Pharmacology and biochemistry transforms
- ☐ User-defined X functions
- ☐ User-defined Y functions

☐ Interchange X and Y (then transform as specified below).

☒ Transform X values using  $X = \text{Log}[X]$  K =

☐ Transform Y values using  $Y = K \cdot Y$  K =

☒ Same K for all data sets. K =

☐ Different K for each data set

Data:  K =

When it is impossible to transform a SD or SEM

- ☒ Erase SD or SEM.
- ☐ Convert to an asymmetric 95% confidence interval.

**Replicates**

- ☒ Transform individual Y values
- ☐ Transform the average of replicates

**New graph**

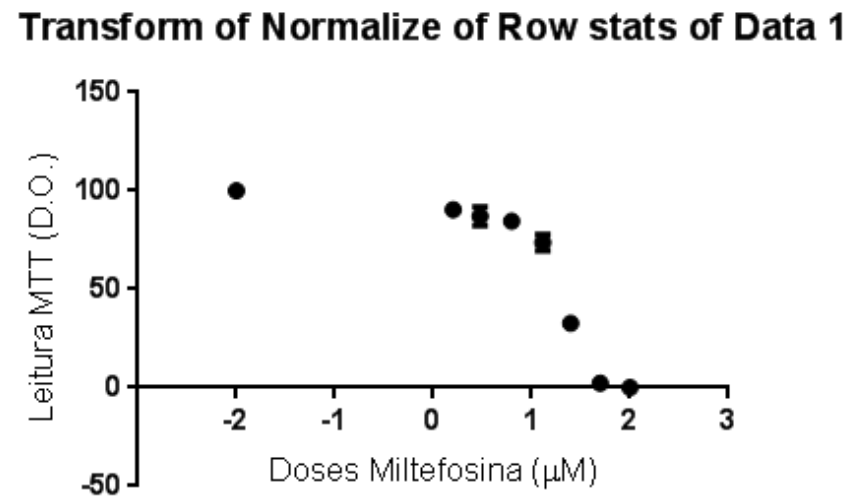
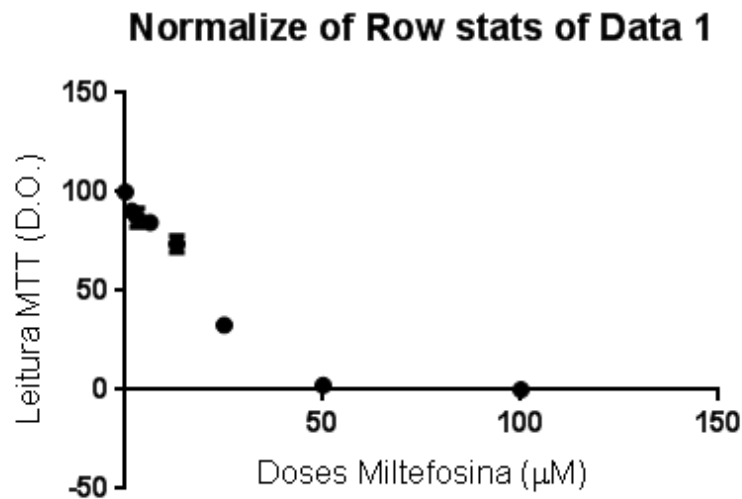
- ☒ Create a new graph of the results

Learn Cancel OK

Normalize of Row stats of Data 1 Normalized data



# Construção de Curva de $CE_{50}$



# Construção de Curva de CE<sub>50</sub>

GraphPad Prism 7.03 - [Project1:Transform of Normalize of Row stats of Data 1]

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

Family  
Search results  
Data with Results  
Data 1  
...Row stats of Data 1  
.....Normalize of Row stats of Data 1  
.....Transform of Normalize of Row stats of Data 1  
Data Tables  
Info  
Project info 1  
Results  
Graphs  
Data 1  
Row stats of Data 1  
Normalize of Row stats of Data 1  
Transform of Normalize of Row stats of Data 1  
Layouts  
Floating Notes

	X	A			B			C			D			E	
	Doses Miltefosina (µM)	Leitura MTT (D.O.)			Title			Title			Title			Title	
	X	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
1	-2.000	100.000	3.705	3											
2	0.204	90.338	3.058	3											
3	0.477	87.055	4.830	3											
4	0.799	84.663	2.629	3											
5	1.114	73.682	4.273	3											
6	1.398	32.670	3.488	3											
7	1.699	2.180	0.599	3											
8	2.000	0.000	0.132	3											
9															
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25															

Transform:  
X=Log(X)

Transform of Normalize of Row stats of Data 1 Transformed data

# Construção de Curva de $CE_{50}$

The screenshot displays the GraphPad Prism 7.03 interface. The 'Analyze Data' dialog box is open, showing the 'Built-in analysis' dropdown and a list of analysis options. The 'Nonlinear regression (curve fit)' option is selected under the 'XY analyses' category. A red arrow points to the 'Analyze' button in the top toolbar. Another red arrow points to the 'Nonlinear regression (curve fit)' option. A third red arrow points to the 'OK' button in the dialog box. A yellow box in the bottom right corner indicates the transformation: 'Transform: X=Log(X)'. The background shows a data table with columns 'Doses Miltefosina (uM)' and 'X'.

	Doses Miltefosina (uM)	X
1	-2.000	
2	0.204	
3	0.477	
4	0.799	
5	1.114	
6	1.398	
7	1.699	
8	2.000	
9		
10		
11		
12		
13		
14		
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20		
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22		
23		
24		
25		

# Construção de Curva de $CE_{50}$

GraphPad Prism 7.03 - [Project1: Transform of Normalize of Row stats of Data 1]

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

results  
ith Results  
ta 1  
...Row stats of Data 1  
.....Normalize of Row stats of Data  
.....Transform of Normalize of Row stats of Data 1  
ables  
ject info 1  
a 1  
w stats of Data 1  
rmalize of Row stats of Data 1  
nsform of Normalize of Row stats of Data 1  
s  
g Notes

	X
1	-2.000
2	0.204
3	0.477
4	0.799
5	1.114
6	1.398
7	1.699
8	2.000
9	
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23	
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25	

Doses Miltefosina (uM)

Parameters: Nonlinear Regression

Fit Compare Constrain Weights Initial values Range Output Confidence Diagnostics Flag

Choose an equation

- ☐ Recently used
  - log(inhibitor) vs. normalized response -- Variable slope
  - Sigmoidal dose-response (variable slope)**
  - log(agonist) vs. response -- Find EC anything
  - Sigmoidal dose-response (variable slope) [2]
  - Michaelis-Menten
  - One phase decay
- ☐ User-defined equations
- ☐ Standard curves to interpolate
- ☐ Dose-response - Stimulation
- ☐ Dose-response - Inhibition
- ☐ Dose-response - Special
- ☐ Binding - Saturation
- ☐ Binding - Competitive
- ☐ Binding - Kinetics
- ☐ Enzyme kinetics - Inhibition
- ☐ Enzyme kinetics - Substrate vs. Velocity
- ☐ Exponential
- ☐ Lines

If you subtracted off the baseline, constrain Bottom to a constant value of 0.0. If you normalized your data, also constrain Top to 100.0

Sigmoidal dose-response (variable slope) [Learn about this equation](#)

Fitting method

☒ Least squares (ordinary) fit ☐ Robust fit ☐ Automatic outlier elimination

Interpolate

☐ Interpolate unknowns from standard curve. Confidence interval: None

Learn Cancel OK

Transform:  
X=Log(X)

# Construção de Curva de CE<sub>50</sub>

GraphPad Prism 7.03 - [Project1:Nonlin fit of Transform of Normalize of Row stats of Data 1]

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

Nonlin fit

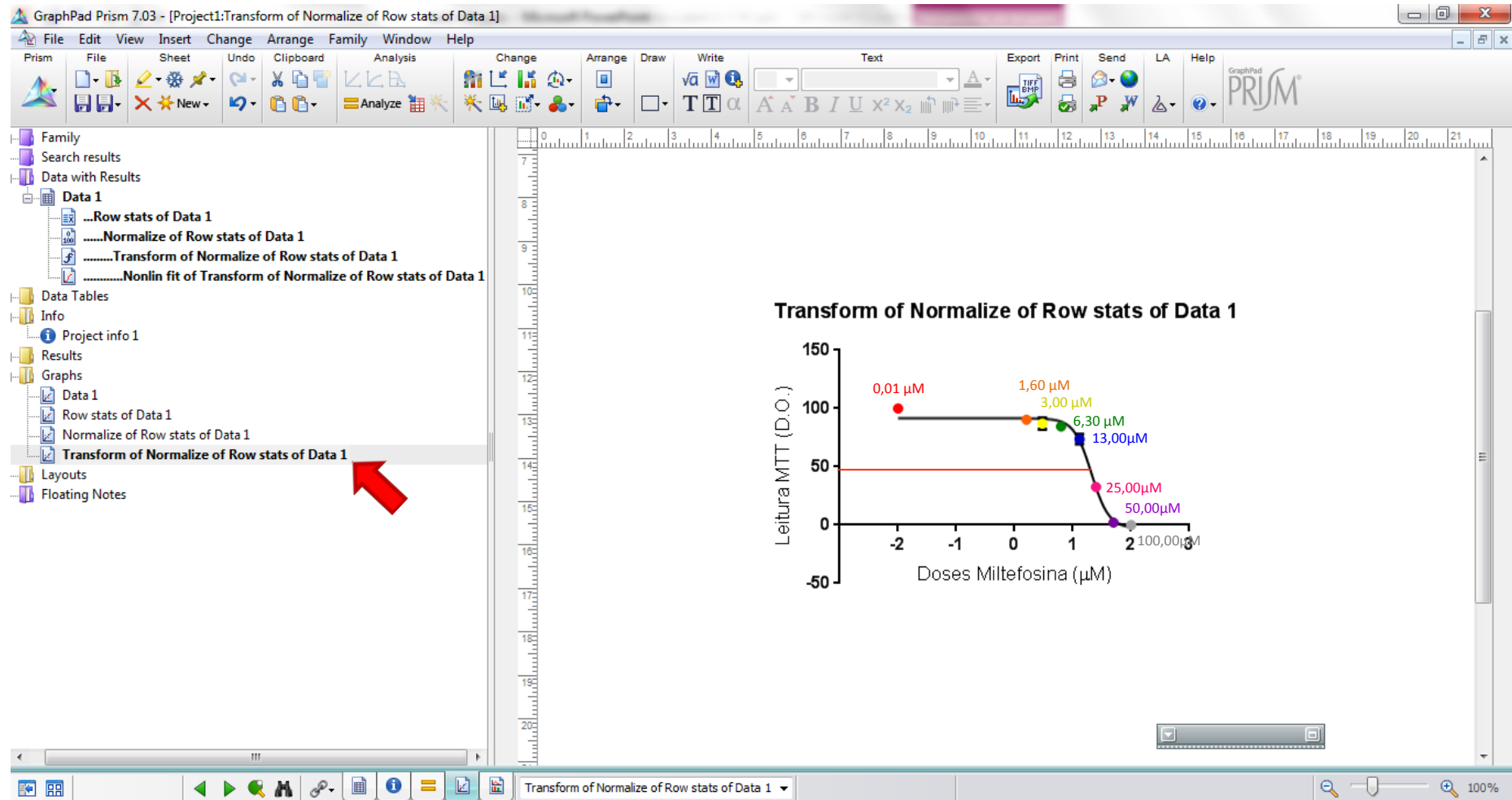
	A	B	C	D	E	F	G	H	I	J	K	L	M
	Leitura MTT (D.O.)	Title	Title	Title	Title	Title	Title	Title	Title	Title	Title	Title	Title
1	Sigmoidal dose-response (variable slope)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
2	Best-fit values												
3	Bottom	-2.652											
4	Top	91.58											
5	LogEC50	1.319											
6	HillSlope	-2.9											
7	EC50	20.86											
8	Std. Error												
9	Bottom	2.912											
10	Top	1.607											
11	LogEC50	0.02136											
12	HillSlope	0.3648											
13	95% CI (profile likelihood)												
14	Bottom	-9.568 to 2.999											
15	Top	88.19 to 95.17											
16	LogEC50	1.276 to 1.364											
17	HillSlope	-3.875 to -2.154											
18	EC50	18.86 to 23.13											
19	Goodness of Fit												
20	Degrees of Freedom	20											
21	R square	0.9855											
22	Absolute Sum of Squares	513.4											
23	Sy.x	5.067											
24													
25	Number of points												

Table of results

Sigmoidal dose-response (variable slope)

Equation help

# Construção de Curva de CE<sub>50</sub>



# Construção de Curva de outras $CE_x$

GraphPad Prism 7.03 - [Project1: Transform of Normalized Row stats of Data 1]

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

Family Search results Data with Results Data 1 ...Row stats of Data 1 ...Normalize of Row stats of Data 1 ...Transform of Normalized Row stats of Data 1 Data Tables Info Project info 1 Results Graphs Data 1 Row stats of Data 1 Normalize of Row stats of Data 1 Transform of Normalized Row stats of Data 1 Layouts Floating Notes

	X	A			B			C			D			E	
	Doses Miltefosina (µM)	Leitura MTT (D.O.)			Title			Title			Title			Title	
	X	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
1	-2.000	100.000	3.705	3											
2	0.204	90.338	3.058	3											
3	0.477	87.055	4.830	3											
4	0.799	84.663	2.629	3											
5	1.114	73.682	4.273	3											
6	1.398	32.670	3.488	3											
7	1.699	2.180	0.599	3											
8	2.000	0.000	0.132	3											
9															
10															
11															
12															
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14															
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16															
17															
18															
19															
20															
21															
22															
23															
24															
25															

Transform:  
X=Log(X)

# Construção de Curva de outras $CE_x$

GraphPad Prism 7.03 - [Project1: Transform of Normalize of Row stats of Data 1]

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

Family

- Search results
- Data with Results
  - Data 1
    - ...Row stats of Data 1
    - .....Normalize of Row stats of Data 1
    - .....Transform of Normalize of Row stats of Data 1
- Data Tables
- Info
  - Project info 1
- Results
- Graphs
  - Data 1
  - Row stats of Data 1
  - Normalize of Row stats of Data 1
  - Transform of Normalize of Row stats of Data 1
- Layouts
- Floating Notes

Doses Miltefosina ( $\mu\text{M}$ )

	X
1	-2.000
2	0.204
3	0.477
4	0.799
5	1.114
6	1.398
7	1.699
8	2.000
9	
10	
11	
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Analyze Data

Built-in analysis

Which analysis?

- Remove baseline and column math
- Transpose X and Y
- Fraction of total
- XY analyses**
  - Nonlinear regression (curve fit)**
  - Linear regression
  - Fit spline/LOWESS
  - Smooth, differentiate or integrate curve
  - Area under curve
  - Deming (Model II) linear regression
  - Column statistics
  - Row means with SD or SEM
  - Correlation
  - Interpolate a standard curve
- Column analyses**
- Grouped analyses**
- Contingency table analyses**
- Survival analyses**
- Parts of whole analyses**
- Generate curve**
- Simulate data**
- Recently used**

Analyze which data sets?

☒ A: Leitura MTT (D.O.)

When you analyze tables or graphs with more than one data set, use this space to select which data set(s) to analyze.

Select All Deselect All

Help Cancel OK

Transform:  
X=Log(X)



# Construção de Curva de outras $CE_x$

GraphPad Prism 7.03 - [Project1: Transform of Normalize of Row stats of Data 1]

Parameters: Nonlinear Regression

Choose an equation

- Recently used
  - log(inhibitor) vs. normalized response -- Variable slope
  - Sigmoidal dose-response (variable slope)
  - log(agonist) vs. response -- Find EC anything
  - Sigmoidal dose-response (variable slope) [2]
  - Michaelis-Menten
  - One phase decay
- User-defined equations
  - Standard curves to interpolate
  - Dose-response - Stimulation
  - Dose-response - Inhibition
  - Dose-response - Special
  - Binding - Saturation
  - Binding - Competitive
  - Binding - Kinetics
  - Enzyme kinetics - Inhibition
  - Enzyme kinetics - Substrate vs. Velocity
  - Exponential
  - Lines

Fits a variable slope log(dose) vs. response curve, but fits the EC80 or EC90... instead of the EC50. You must constrain F to a constant value between 0 and 100. For example, if you want to fit the EC80, constrain F to equal 80.  
log(agonist) vs. response -- Find EC anything

Fitting method

☒ Least squares (ordinary) fit ☐ Robust fit ☐ Automatic outlier elimination

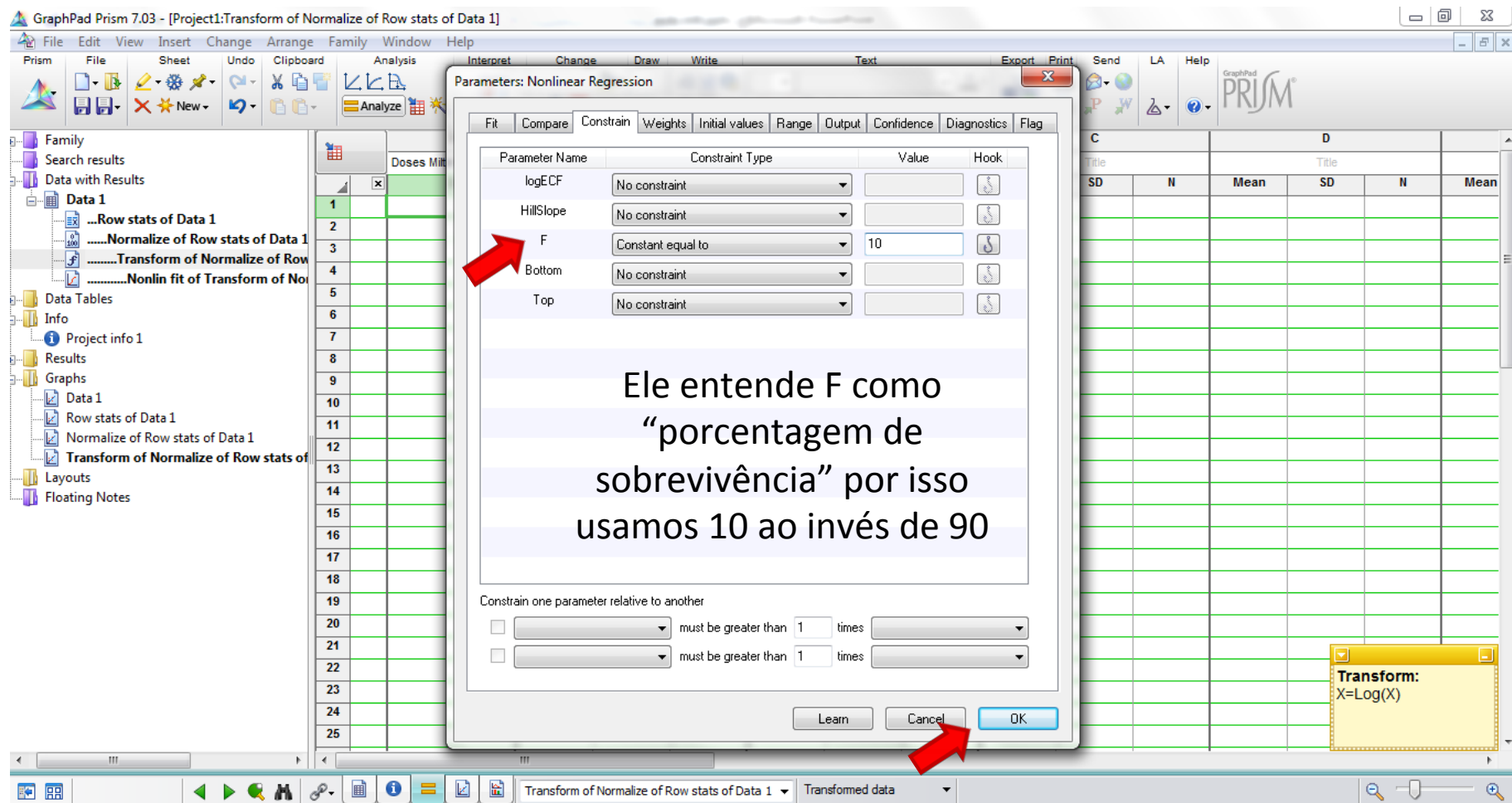
Interpolate

☐ Interpolate unknowns from standard curve. Confidence interval: None

Learn Cancel OK

Transform:  
X=Log(X)

# Construção de Curva de outras $CE_{90}$



GraphPad Prism 7.03 - [Project1: Transform of Normalize of Row stats of Data 1]

Parameters: Nonlinear Regression

Parameter Name	Constraint Type	Value	Hook
logECF	No constraint		
HillSlope	No constraint		
F	Constant equal to	10	
Bottom	No constraint		
Top	No constraint		

Ele entende F como “porcentagem de sobrevivência” por isso usamos 10 ao invés de 90

Constrain one parameter relative to another

☐ must be greater than 1 times

☐ must be greater than 1 times

Learn Cancel OK

Transform:  
X=Log(X)

# Construção de Curva de outras $CE_{90}$

GraphPad Prism 7.03 - [Project1:Nonlin fit of Transform of Normalize of Row stats of Data 1]

File Edit View Insert Change Arrange Family Window Help

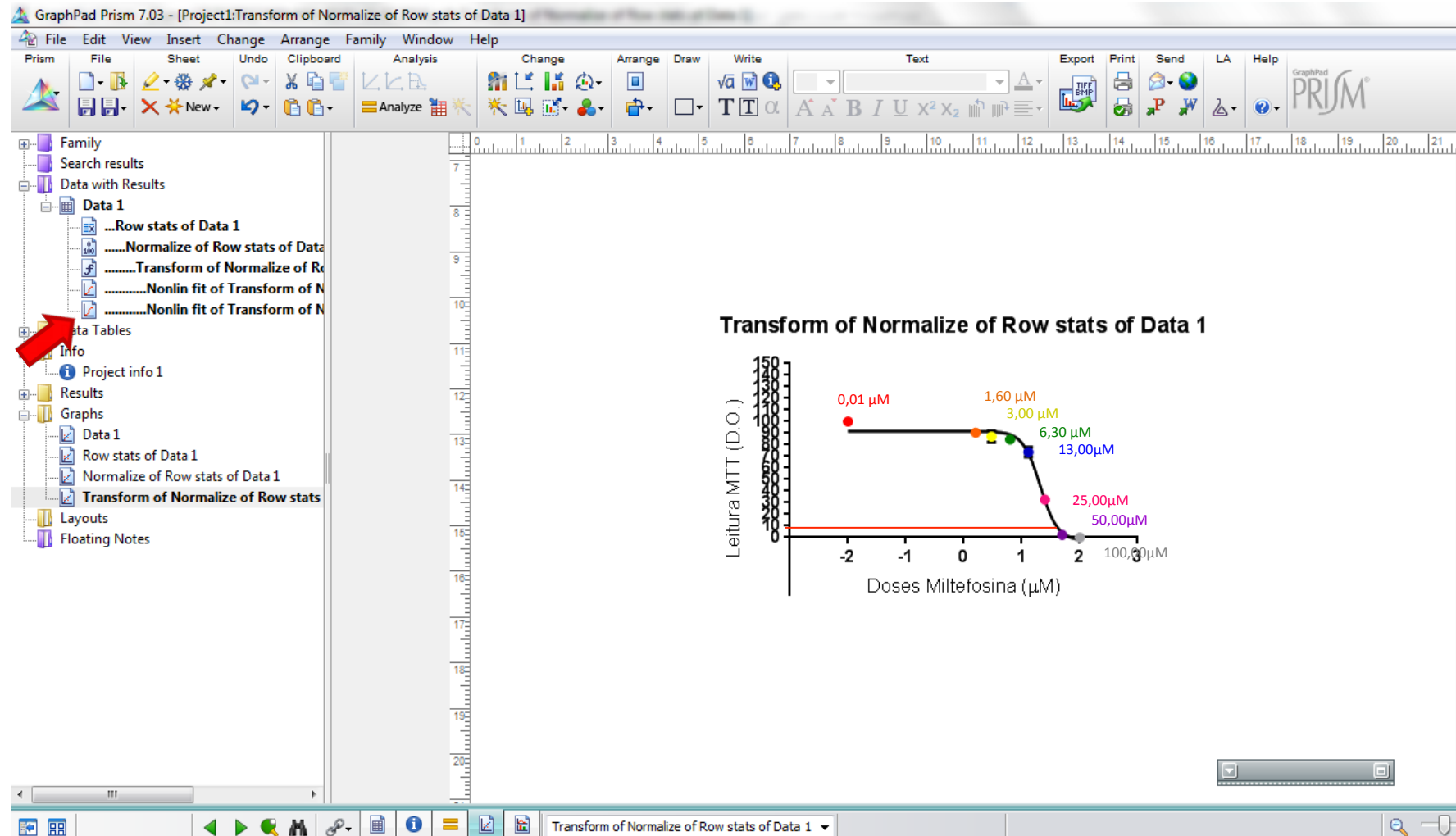
Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

results  
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.....Normalize of Row stats of Data 1  
.....Transform of Normalize of Row stats of Data 1  
.....Nonlin fit of Transform of Normalize of Row stats of Data 1  
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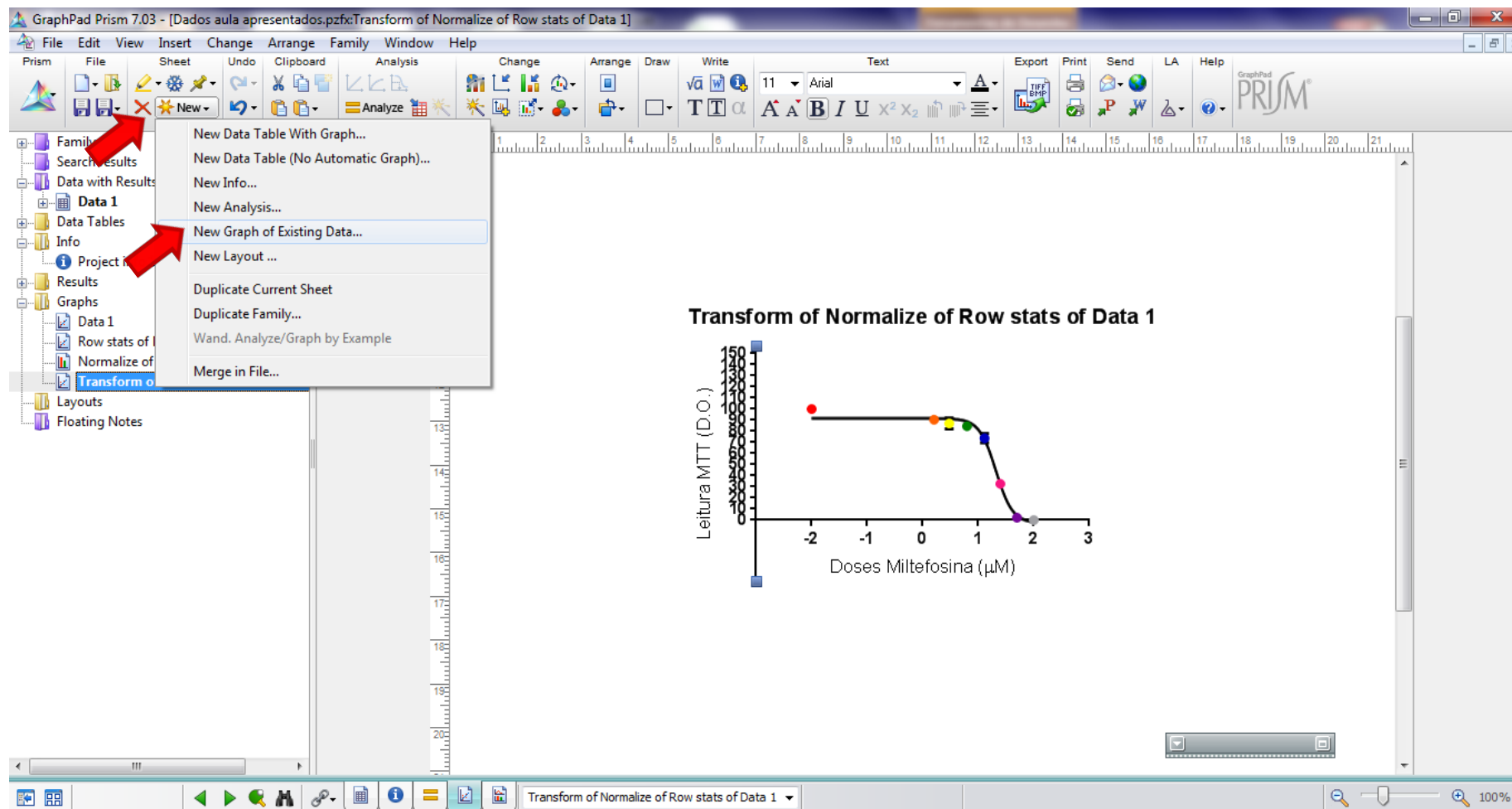
Nonlin fit		A	B	C	D	E	F	G	H	I
		Leitura MTT (D.O.)	Title	Title	Title	Title	Title	Title	Title	Title
1	log(agonist) vs. response -- Find ECanything	Y	Y	Y	Y	Y	Y	Y	Y	Y
2	Best-fit values									
3	logECF	1.648								
4	HillSlope	-2.9								
5	F	= 10								
6	Bottom	-2.652								
7	Top	91.58								
8	ECF	44.5								
9	Span	94.24								
10	Std. Error									
11	logECF	0.05036								
12	HillSlope	0.3648								
13	Bottom	2.912								
14	Top	1.607								
15	Span	3.539								
16	95% CI (profile likelihood)									
17	logECF	1.556 to 1.782								
18	HillSlope	-3.875 to -2.154								
19	Bottom	-9.568 to 2.999								
20	Top	88.19 to 95.17								
21	ECF	35.95 to 60.53								
22	Goodness of Fit									
23	Degrees of Freedom	20								
24	R square	0.9855								

Nonlin fit of Transform of Normalize of Row sta Table of results

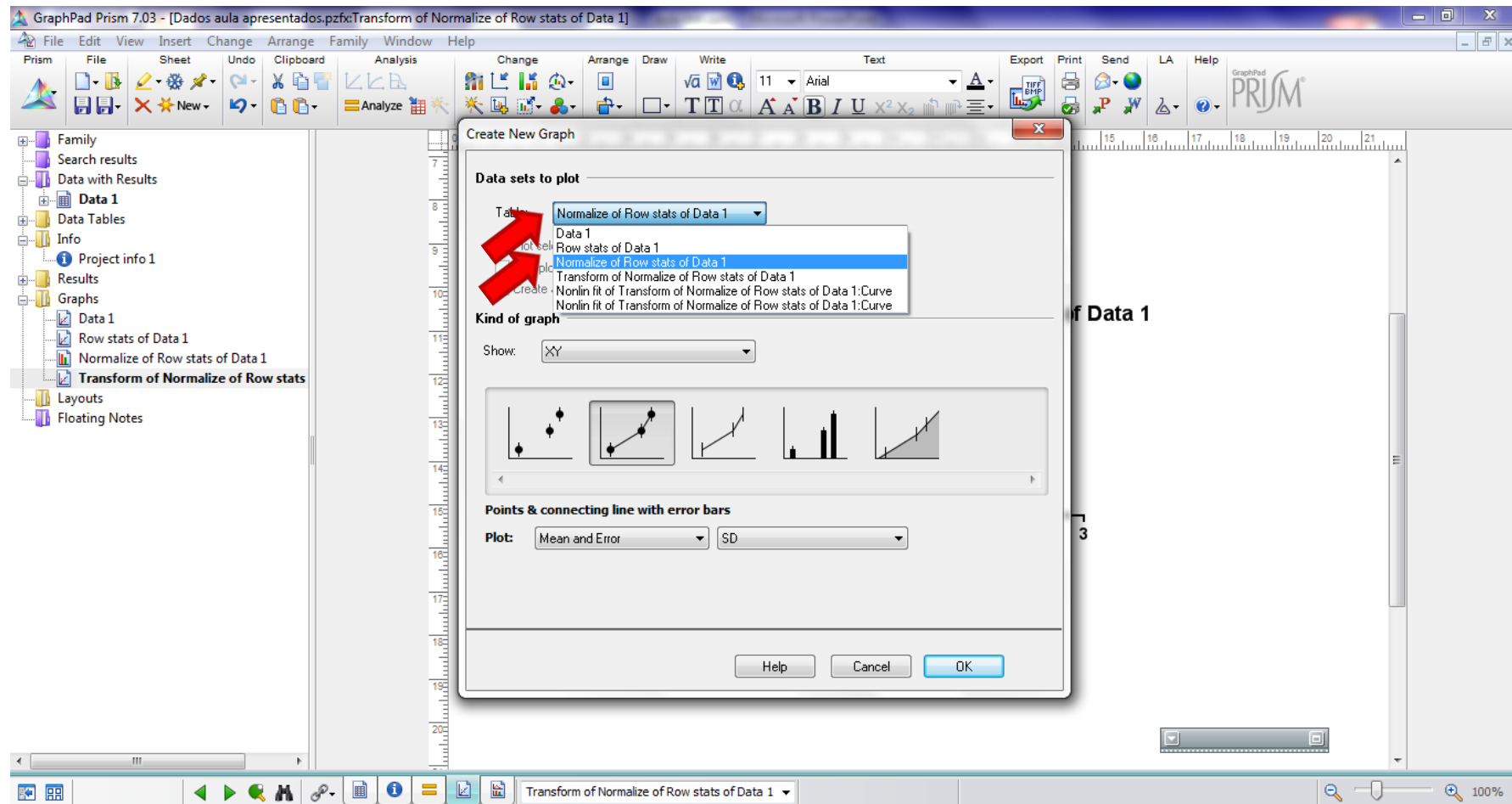
# Construção de Curva de outras $CE_{90}$



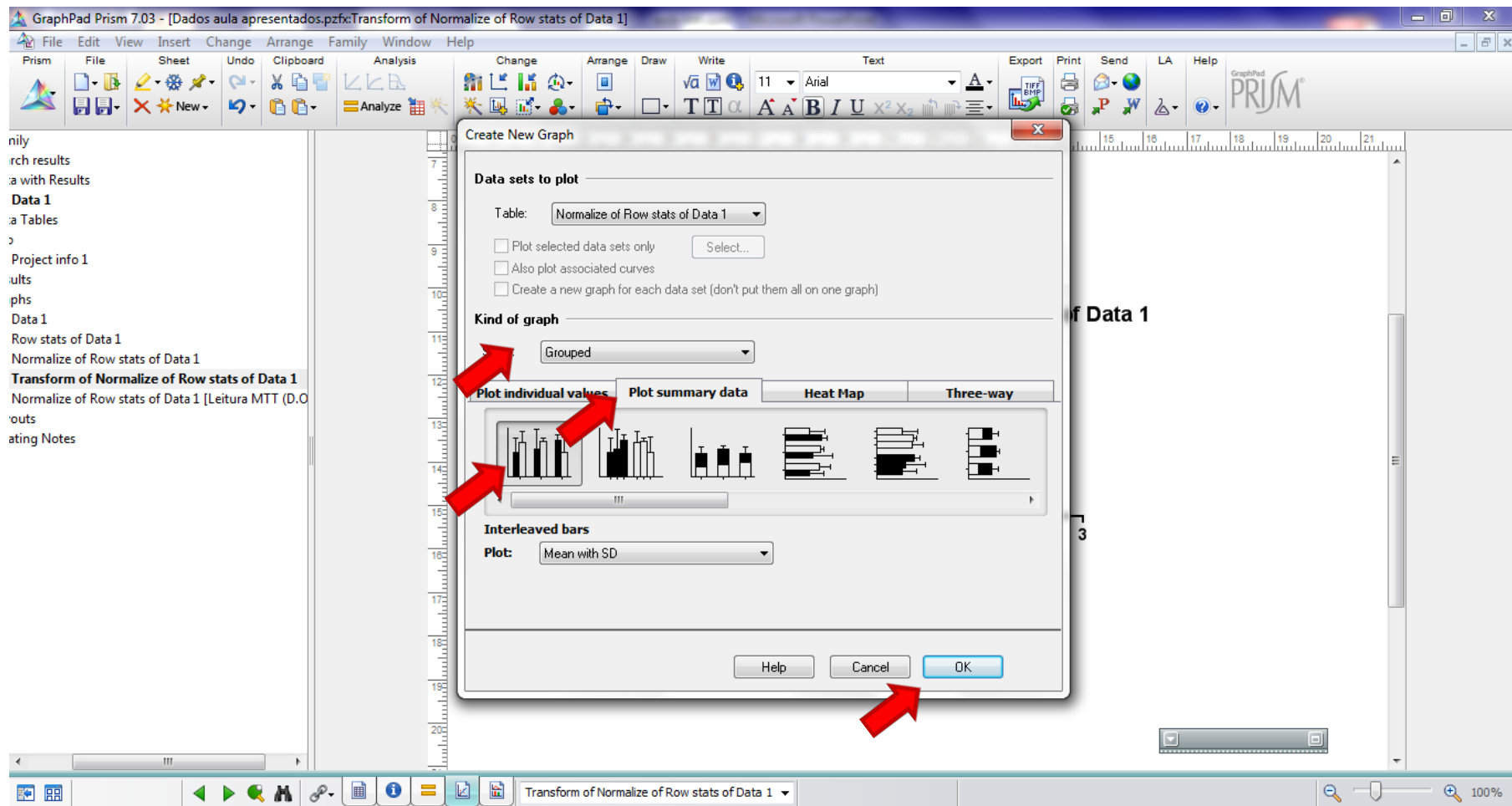
## Observando os resultados de outras formas:



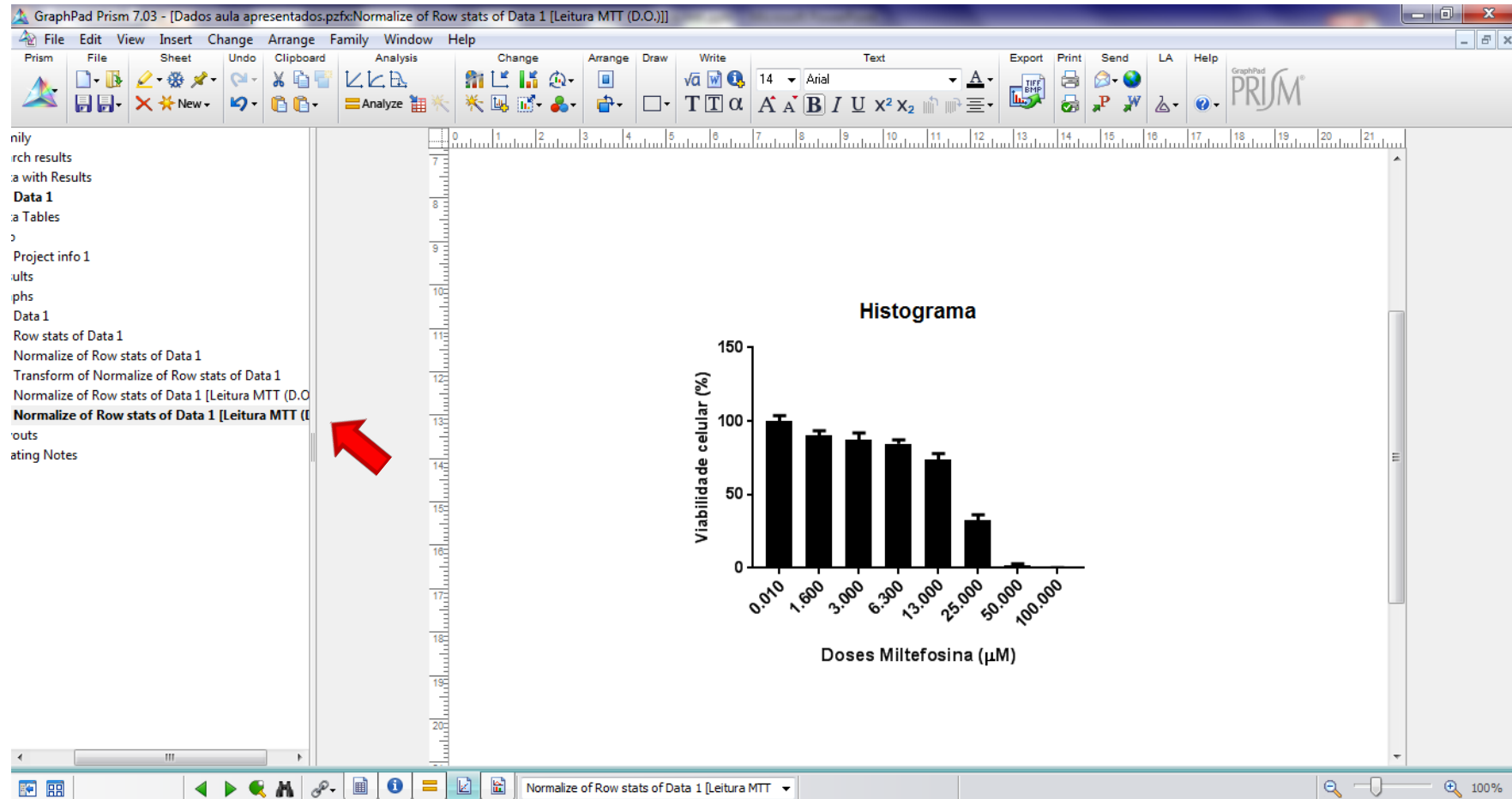
# Observando os resultados de outras formas:



# Observando os resultados de outras formas:



# Observando os resultados de outras formas:





# Atividade prática

- Cada grupo (5 alunos) receberá uma folha contendo as DOs referentes a um ensaio de MTT com isolados de *L. braziliensis* tratados com miltefosina ou anfotericina B
- Cada grupo deverá:
  - Construir um gráfico de histograma (média e desvio padrão) mostrando a % de viabilidade em cada concentração de fármaco.
  - Construir as curvas de  $CE_{50}$  e  $CE_{90}$  no programa Graph Prism 7.0.
  - Calcular o valor de  $CE_{50}$  e  $CE_{90}$  dos seus experimentos.
  - Comparar os resultados.