IVART Online Step-by-step User guide

Version 1.0

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1. Scope

The In Vitro Analysis and Reporting Tool (IVART) is an application developed by WWARN to generate reproducible IC50 estimates from *in vitro* malaria drug sensitivity assays. Data from any *in vitro* growth inhibition assay method can be analysed, including HRP2 ELISA, pLDH ELISA, isotope uptake inhibition, SYBR® Green, and the WHO micro-test (mark III).

2. Technical requirements

Internet Browser
- Internet Explorer 9 or higher
- Mozilla Firefox 13 or higher
- Google chrome 25 or higher
- Safari 5 or higher

Excel 2007, 2010 or updates for 2003 where .xlsx files can be opened and saved.

3. Log in to the IVART site

Go to https://www.wwarn.org/toolkit/data-management/ivart and follow the instructions to access the tool. Log in to the site using your WWARN user name and password. If you do not have a WWARN account, please register: https://www.wwarn.org/user/register .

When entering the IVART page, click *Next* to follow the tutorials or *Use IVART* to go directly to the analysis.

4. Preparation of data files to upload

Data for IVART online processing must:
- be organised in a 96-well plate format with 8 horizontal rows and 12 vertical columns.
- be annotated by a system of "Tags" to identify results plates and drug layouts (section 4.1).
- include at least two drug-free controls on each plate
- be saved in Microsoft Excel 2007 format (.xlsx)

Files ready for upload to IVART can be generated in two ways:
1) Copy & Paste data into the IVART Data Template (section 4.2). Not only is this an easy solution that allows you to quickly get started with your analysis, but it is helpful in organising data for prospective studies.
2) Add tags to identify drug concentration layouts and result plates in your own data files (section 4.3). Users who become familiar with this more complex approach can process large data sets more quickly.

4.1 IVART Tags

"Tags" are strings of text placed in the cell immediately below and to the right of the cells corresponding to a 96-well plate of results or drug concentration data. Tags are critical to defining the location of a plate and ensure that results are matched correctly with drugs and concentrations. Result tags contain information about samples and the study (Table 1).

Table 1: Variables in the Results Plate Tag

Variable in the result tag	Description	Value
ID1	Sample ID1	Required
ID2	Sample ID2	Optional
Date	Date of sampling from patient (dd/mm/yyyy)	Optional
DrugLayout	Name of Drug concentration layout	Required
Country	Country patient infected	Optional
StudySiteName	Location of study	Optional
Method	Readout method + duration of test	Optional
Lat	Latitude	Optional
Lon	Longitude	Optional
SampleType	Reference clone	Only required for references; leave blank for field isolates
Batch	Date of preparation of drug plate batch (dd/mm/yyyy)	Optional

4.2 Using the IVART data template

Complete all three Microsoft Excel-based IVART Data Template worksheets

4.2.1 Drug Concentration Layouts worksheet

a. Format "drug: drug concentration in nM" (e.g. "LUM:10").
b. Format drug-free control as "drug:0". At least two drug-free controls are required.
c. Drugs with μM range inhibitory levels (e.g. cyclins) may be reported in μM. Keep this in mind when reviewing the analysis.
d. Download drug abbreviations used by WWARN.
e. Only white cells can be changed; the other cells are locked.
f. Overwrite the examples in the white cells with your own drug concentration layout in any format - by column or row, with increasing or decreasing concentrations.
g. Enter decimals with a comma"," or a full stop".". All commas will be replaced with full stop in the IVART data transformation process.

h. Write a unique name for each layout in the white cell, e.g. A, B, etc. The template automatically generates a drug layout tag seen in the dark blue cell used by IVART to extract data.

i. Include up to 10 uniquely-named layouts, if necessary. For more than 10 layouts, create a second copy of the *Drug Concentration Layout* worksheet in the Data Template file.

j. Leave empty wells blank.

Example 1: A drug concentration layout showing the unique layout name (white cell) and corresponding tag (dark blue cell, lower right)

4.2.2 Results Isolates worksheet

a. Use the *Results Isolates* worksheet to enter the test results (counts per minute, absorbance, microscope count, etc.) in a 96-well format.

b. Data can only be entered in the white cells; the other cells are locked.

c. Overwrite the sample results in the white plate outline by copy/pasting your isolate test results (see Example 2).

d. Enter sample and study information into the white cells of the red (required) and blue (optional) variables immediately to the right of each plate. The template will automatically generate the plate tag (dark blue, lower right) – see Example 3.

e. Use cells R6:X6 (Example 3) to enter default values for the entire data set, which will be added to the plate tag if no plate-specific values are entered, e.g. in cells R10:X10.

f. Enter dates in the format dd/mm/yyyy. If that format is not supported in your regional version of Excel, write the date in your regional format and make sure that the date is captured in the dd/mm/yyyy format in the tag (dark blue) – see Example 3.

g. Blank cells will not be analysed.

h. Each *Results Isolates* worksheet has 150 plate outlines. To analyse more plates, create a copy of the *Results Isolates* worksheet in the template file.

Example 2: Entering results from a 96-well plate assay into the *Results Isolates* worksheet

Example 3: Additional sample or study information.

4.2.3 Results References worksheet

    a. Use the *Results References* worksheet to enter reference clone data, which IVART will analyse separately.

    b. In addition to the *Sample ID1* and *Drug Layout* variable, IVART requires *Sample Type* information (red), used to identify different reference clones, e.g. 3D7, W2, HB3, etc. Any text may be used, provided there is consistency between reference clones to be analysed together.

    c. IVART uses the *Sample ID1, Sample ID2,* and *Date* variables to identify and perform separate analyses of plates. If the reference has been tested at several times or in replicates, it is important to make sure that the identifier composed by these three variables is unique for each plate, so they can be identified as separate assays by IVART.
Example:
ID1: 3D7, *ID2*: A, *Date*: 29/03/2007
ID1: 3D7, *ID2*: B, *Date*: 29/03/2007

4.3 Adding tags to identify drug concentration layouts and result plates in your own data files

If your data and sample variables are already organised in an Excel file, you can create tags to annotate the data and the drug concentrations for IVART analysis directly in your file. Use the sample Excel tagging file to copy the tags.

Each set of drug concentration data or results corresponding to a single 96-well plate must have a tag - a string of variables identifying the layout, sample, and study data, which IVART will used to extract appropriate information for analysis. See section 4.1 for further information.

Tags are placed in the cell immediately below and to the right of the 96-well plate (see Examples 4 and 5).

4.3.1 Drug concentration layouts

a. Create a drug concentration layout worksheet following the sample tagging file
b. Enter drug concentrations in the format "drug: drug concentration in nM" (e.g. "LUM:10") in any format - by column or row, with increasing or decreasing concentrations.
c. *At least two* drug-free controls are required in the format "drug:0".
d. Create a tag in the lower right corner of each plate (cell O11, Example 4) in the format "DRUG-LAYOUT:NAME" (e.g. "DRUG-LAYOUT:A"), which IVART will use to extract drug concentration values.
e. Unused wells may be left blank.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Unit nM															
2			1	2	3	4	5	6	7	8	9	10	11	12		
3		A	LUM:0	LUM:0	CQ:0	CQ:0	AQ:0	AQ:0	DQ:0	DQ:0	MQ:0	MQ:0	DHA:0	DHA:0		
4		B	LUM:1.2	LUM:1.2	CQ:50	CQ:50	AQ:5	AQ:5	DQ:5	DQ:5	MQ:2.5	MQ:2.5	DHA:0.25	DHA:0.25		
5		C	LUM:2.4	LUM:2.4	CQ:100	CQ:100	AQ:10	AQ:10	DQ:10	DQ:10	MQ:5	MQ:5	DHA:0.5	DHA:0.5		
6		D	LUM:4.8	LUM:4.8	CQ:200	CQ:200	AQ:20	AQ:20	DQ:20	DQ:20	MQ:10	MQ:10	DHA:1	DHA:1		
7		E	LUM:10	LUM:10	CQ:400	CQ:400	AQ:40	AQ:40	DQ:40	DQ:40	MQ:20	MQ:20	DHA:2	DHA:2		
8		F	LUM:19	LUM:19	CQ:800	CQ:800	AQ:80	AQ:80	DQ:80	DQ:80	MQ:40	MQ:40	DHA:4	DHA:4		
9		G	LUM:39	LUM:39	CQ:1600	CQ:1600	AQ:160	AQ:160	DQ:160	DQ:160	MQ:80	MQ:80	DHA:8	DHA:8		
10		H	LUM:78	LUM:78	CQ:3200	CQ:3200	AQ:320	AQ:320	DQ:320	DQ:320	MQ:160	MQ:160	DHA:16	DHA:16		
11															DRUG-LAYOUT:A	
12																
13			1	2	3	4	5	6	7	8	9	10	11	12		
14		A	CQ:0	CQ:6.25	CQ:12.5	CQ:25	CQ:50	CQ:100	CQ:200	CQ:400	CQ:800	CQ:1600	CQ:3200	CQ:0		
15		B	CQ:0	CQ:6.25	CQ:12.5	CQ:25	CQ:50	CQ:100	CQ:200	CQ:400	CQ:800	CQ:1600	CQ:3200	CQ:0		
16		C	MQ:0	MQ:2	MQ:4	MQ:8	MQ:16	MQ:32	MQ:64	MQ:128	MQ:256	MQ:512	MQ:1024	MQ:0		
17		D	MQ:0	MQ:2	MQ:4	MQ:8	MQ:16	MQ:32	MQ:64	MQ:128	MQ:256	MQ:512	MQ:1024	MQ:0		
18		E	DQ:0	DQ:3.75	DQ:7.5	DQ:15	DQ:30	DQ:60	DQ:120	DQ:240	DQ:480	DQ:960	DQ:1920	DQ:0		
19		F	DQ:0	DQ:3.75	DQ:7.5	DQ:15	DQ:30	DQ:60	DQ:120	DQ:240	DQ:480	DQ:960	DQ:1920	DQ:0		
20		G	DHA:0	DHA:0.125	DHA:0.25	DHA:0.5	DHA:1	DHA:2	DHA:4	DHA:8	DHA:16	DHA:32	DHA:64	DHA:0		
21		H	DHA:0	DHA:0.125	DHA:0.25	DHA:0.5	DHA:1	DHA:2	DHA:4	DHA:8	DHA:16	DHA:32	DHA:64	DHA:0		
22															DRUG-LAYOUT:B	
23																

Example 4: Two drug concentration layouts

4.3.2 Tagging of result plates

a. Copy the tag from the sample tagging file (cell N12, Results worksheet) and paste it in the corresponding position relative to the results on the first plate (See N12 in Example 5).

b. The tag references information about the sample and study, which it finds in cells O4:W4 (see Example 5) or equivalent.

c. If you need to fill in information about each plate in your date file, for guidance, cut and paste the headers O1:Y1 from the sample tagging file to a corresponding position in your own file.

d. If your data file contains information about each plate, see section 4.3.3 on how to adapt the tag to your specific data set.

e. Copy and paste the tag next to every plate in the data file and fill in the required information in the cells to which the tag refers. The tag value changes automatically as information is provided.

f. The format of dates depends on you regional version of Excel. Fill in the date in a suitable format for your version and make sure that the date appears in the format dd/mm/yyyy in the tag (Example 5). Tagging errors are often due to incorrectly formatted dates.

g. The *Sample Type* variable is used to distinguish reference clones from field isolates. Text may be entered in any format (e.g. 3D7, 3d7, W2, HB3, etc.), but must be consistent for reference clones of a particular type to be analysed together. Leave cells blank for field isolates.

h. Result plates may be provided on one or several worksheets.

i. Data from corresponding results cells left blank in the drug layout (e.g. columns 11-12,) will not be analysed. If appropriate, ensure that cells (e.g. plate column 11-12, Example 6) are blank in the corresponding result plates and that the tag is correctly placed.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
1															Sample ID1*	Sample ID2	Date of sampling from patient (dd/mm/yyy	Drug Layout*	Country patient infected	Study Site name	Method - Readout	Latitude	Longitude	Sample Type - Reference clone
2																								
3		1	2	3	4	5	6	7	8	9	10	11	12											
4	A	0.662	0.659	0.692	0.599	0.649	0.666	0.678	0.688	0.635	0.602	0.596	0.542		ABC	1	2007-03-22	A	Kenya	Kisumu	HRP2 72 hr	-0.074844	34.766808	
5	B	0.621	0.663	0.439	0.494	0.687	0.66	0.645	0.625	0.508	0.567	0.427	0.492											
6	C	0.599	0.625	0.371	0.489	0.617	0.659	0.689	0.654	0.593	0.709	0.5	0.492											
7	D	0.362	0.344	0.297	0.336	0.33	0.371	0.599	0.515	0.497	0.686	0.337	0.352											
8	E	0.312	0.372	0.261	0.275	0.313	0.304	0.586	0.572	0.434	0.509	0.195	0.213											
9	F	0.265	0.285	0.256	0.254	0.223	0.206	0.553	0.551	0.379	0.347	0.179	0.153											
10	G	0.21	0.243	0.293	0.267	0.175	0.193	0.297	0.291	0.252	0.268	0.13	0.134											
11	H	0.202	0.209	0.282	0.261	0.178	0.195	0.224	0.218	0.172	0.189	0.116	0.114											
12															RESULT-PLATE-ID1:ABC|ID2:1|Date:22/3/2007|DrugLayout:A|Country:Kenya|StudySiteName:Kisumu|Method:HRP2 72 hr|Lat:-0.0748									
13																								

Example 5: Generating a tag for a set of isolate results

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
14		1	2	3	4	5	6	7	8	9	10	11	12							
15	A	0.675	0.672	0.705	0.612	0.662	0.679	0.691	0.701	0.655	0.615				ABC	2	2007-03-29	A	Kenya	Kisumu
16	B	0.634	0.676	0.452	0.507	0.7	0.673	0.658	0.638	0.521	0.58									
17	C	0.612	0.638	0.384	0.502	0.63	0.672	0.702	0.667	0.606	0.722									
18	D	0.375	0.357	0.31	0.349	0.343	0.384	0.612	0.528	0.51	0.699									
19	E	0.325	0.385	0.274	0.288	0.326	0.317	0.599	0.585	0.447	0.522									
20	F	0.278	0.298	0.269	0.267	0.236	0.219	0.566	0.564	0.392	0.36									
21	G	0.223	0.256	0.306	0.28	0.188	0.206	0.31	0.304	0.265	0.281									
22	H	0.215	0.222	0.295	0.274	0.191	0.208	0.237	0.231	0.185	0.202									
23															RESULT-PLATE-ID1:ABC|ID2:2|Date:29/3/2007|DrugLayout:A|Country:Kenya|St					
24																				

Example 6: Empty wells need no further work

4.3.3 Changing the tag

a. Tag functions may be edited, for example, to change the location of a cell from which a variable is retrieved, or to enter the value of a variable constant throughout a data set. Always copy a tag from an Excel spreadsheet and make changes within the programme.

b. Click on the tag to view the function. In the tag from Example 7, variables are shown in bold; the cell locator from which a value is retrieved appears in colour.
   ="RESULT-PLATE-ID1:"&O15&"|ID2:"&P15&"|Date:" & DAY(Q15) & "/" & MONTH(Q15) & "/" & YEAR(Q15) & "|DrugLayout:"&R15&"|Country:"&S15&"|StudySiteName:"&T15&" |Method:"&U15&"|Lat:"&V15&"|Lon:"&W15&"|SampleType:"&X15&"|Batch:" & DAY(Y15) & "/" & MONTH(Y15) & "/" & YEAR(Y15) & ""

c. To change according to the cells where the information is located, *Sample ID1* of a plate should be retrieved from cell A4 instead of O4.
   ="RESULT-PLATE-ID1:"&O15&"| → ="RESULT-PLATE-ID1:"&A15&"|

d. To make a variable constant throughout a data set, overwrite the reference (highlighted in yellow) with the constant. For example,
   Country:"&S4&"| → Country:Kenya|

e. Variables *ID1* and *DrugLayout* must be provided. However, if there is no information for an optional variable, the reference may be removed or left blank.
   |Lat:"&V4&"|Lon:"&W4&"| → |Lat:|Lon:|

f. To apply changes made to the first plate tag to all plates in the data set, copy the changed tag and paste it next to all subsequent plates.

Example 7: Inside a tag

5. Data Upload

- Register (new user) or Log in to the WWARN website.
- Launch the IVART Online application.
- Click *Next* to follow a simple tutorial or *Use IVART* to upload your prepared, tagged file. *Select* your file, read, and tick the box to show you accept the Terms of Use and

then *Upload.* Your file will be securely stored on WWARN's server. Only you have access to your data.

- The *Plate Assistant* provides feedback on any tagging errors, helping IVART to extract data correctly. It highlights any problems with file organisation or formatting that must be resolved before the file can be analysed and it issues warnings of inconsistencies in the data file. Further details about Plate Assistant messages may be found in the Appendix.
- Holding the cursor over an uploaded file reveals details, including the numbers of drug concentration layouts and result plates. Ensure that this information is consistent with your expectation before proceeding to analysis.
- Delete files by selecting and clicking *Delete selected files.*
- Uploaded files are kept on the WWARN server for one month and are subsequently deleted.

6. Data analysis

- *Select* the uploaded file for analysis. Provide a report name and then click *Analyse Data.* On completion, select the zip file and extract the *InVitroReport.pdf* and *IC50.csv* files. The In Vitro Report includes IC_{50} summary statistics per drug per year, with separate presentations of field sample and reference isolate data, and graphical representation of individual assays. The *IC50.csv* file is a spreadsheet of sample data and IC_{50} parameters. Data analysis methodology is described in the In Vitro Data Management and Statistical Analysis Plan.
- The application does not store your zip file. Download and store the zip file before starting a new analysis or leaving the application.

7. Sharing data with WWARN

Working together to share knowledge and resources, we can help to understand and prevent the spread of malaria drug resistance. Read how sharing your data contributes to these goals. If you would like to proceed, before uploading files to our secure Data Repository, click on *Share selected files* to read and accept extended Terms of Submission, which explain how you may use the WWARN site to submit data and how WWARN will use that data.

8. Support

Contact IVART@wwarn.org for support.

Appendix

Plate Assistance error messages

TYPE	CAUSE	CONSEQUENCE	SOLUTION
DRUG-LAYOUT error	the drug-layout has no name	the drug-layout is nonexistent for IVART	fill cell next to drug-layout as indicated
DRUG-LAYOUT error	the drug-layout has a name that is already used by another drug-layout	the drug-layout is nonexistent for IVART	change drug-layout name to a unique one
SEVERE TAG error	drug-layout used in the tag does not exist	the result plate will not be read by IVART	complete the tag of the result plate with a drug-layout name that was previously declared
SEVERE TAG error	ID1 of the result plate is not unique	the result plate will not be read by IVART	fill the ID1 cell of the corresponding result plate with a unique ID1 identifier
TAG error	a tag was not recognised	the tag for the result plate will be incomplete	verify the spelling of tag
TAG error	a tag was not recognised, tag or tag-value error	the tag for that result plate will be incomplete	verify the syntax of the tag (column, separators...)
TAG error	a duplicate result plate was found but with different location information	tag information is inconsistent	verify existing result plates for tag information consistency (country tag)
DRUG-LAYOUT warning	no concentration could be read by IVART in the drug-layout cell	information in corresponding cells in result plates referring to this drug-layout will not be read by IVART	verify if the cell should be empty or add concentration in drug-layout in the correct format
DRUG-LAYOUT warning	missing ":" in drug-layout concentration cell	information in corresponding cells in result plates referring to this drug-layout will not be read by IVART	add concentration in drug-layout in the correct format
RESULT-PLATE warning	a comma "," was detected in cell value	comma should be automatically changed into a dot "." separator. However multiple "," could provoke more serious errors	change "," separators for numerical values into dot "." separation
RESULT-PLATE warning	a cell was considered empty	cell value will not be included in the analyses	verify if the cell should be empty or check the format of the cell value (does the cell include comma separator or symbols?)
WARNING (date)	a date was detected to be in the future	information and report will have inconsistency	add correct dates