Document Number: **SASoM/METHOD/054.v5**

Title: **Assessing cell growth Additivity / Synergy / Antagonism using 'CalcuSyn' software**

Version: **v5**

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SOP History		
Number	Date	Reason for Change
v1	22/04/2015	Original
v2	22/04/2017	Update
v3	17/08/2017	Update
V4	17/08/2019	Update
V5	09/09/2021	Update

1.0 Purpose –

This SOP describes the current procedure for assessing cell growth additivity / synergy / antagonism using 'CalcuSyn' software in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff in the SASoM using CalcuSyn software.

3.0 Responsibilities –

All staff involved in cell culture are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

CalcuSyn is a piece of software designed to carry out Dose Effect Analysis – usually on cell culture-based growth inhibition studies. A single-user licenced copy of the software is currently installed on the 'dry' pc outside the microscope room (248:0). A paper copy of the manual is kept in the vicinity of the pc.

The software is designed to give a Combination Index (CI) based on multiple drug-effects. This Combination index can be used to indicate Synergism (where CI < 1), Additivity (where CI = 1) or Antagonism (where CI > 1).

Whether an effect is *truly* synergistic depends on a number of factors, including (i) accuracy of measurement and biological activity, (ii) whether such synergism is apparent at all doses / combinations, (iii) experimental conditions such as temperature, hypoxia, pH etc, (iv) whether the treatment schedule is taken into account, and (v) whether we use a constant or non-constant combination ratio.

The following table shows the recommended symbols and descriptions for presenting the degrees of synergism or antagonism:

Range of CI	Symbol	Description
<0.1	+ + + + +	Very strong synergism
0.1 – 0.3	+ + + +	Strong synergism
0.3 -0.7	+ + +	synergism
0.7 – 0.85	+ +	Moderate synergism
0.85 – 0.90	+	Slight synergism
0.90 -1.10	+/-	Nearly Additive
1.10 – 1.20	-	Slight antagonism
1.20 – 1.45	- -	Moderate antagonism

Creating a New Experiment:

Open the CalcuSyn software.

Use the **File / New** command to create a new experiment. The left hand panel will then be populated by the experiment sub-headings. Double click on the **Experiment Details** to enter information regarding the design of the experiment etc. Double click on **Raw Data** to open the Raw Data Grid into which raw data can be stored (athough this is not strictly necessary, it can be useful to have all the data in a single table). You can then save an experiment using the **File / Save** option. All of these options can be accessed via the hotkeys at the top of the screen.

There are two methods to enter a data for any given drug, namely (1) 'Add Drug Wizard', or (2) 'Add Drug Manually'.

In order to use the Add Drug Wizard, all of the data must first be entered into the **Raw Data Grid** in the correct format, as shown below.

	A	B	C	D	E	F	G
1	Drug A		Drug B		Drug A+B		
2	Dose	Effect	Dose	Effect	Dose (A)	Dose (B)	Effect
3	8	1e-007	111	0.016	8	125	0.04109846283164
4	12	0.1961566320006	167	0.06352556134813	12	125	0.1673984386876
5	18	0.4661402677302	250	0.1469028606175	18	125	0.5750565595686
6	27	0.6665891673895	375	0.7205644331971	27	125	0.7053268830089
7	40.5	0.9184379722612	562.5	0.8768029759722	40.5	125	0.7759346859938
8	60.75	0.869613427644			60.75	125	0.8447182752238
9							

Note that all growth data must be presented as 'Effects' and therefore where a drug concentration has no effect the value will be zero (in actual fact you must enter a real number such as 0.0000001 in order that the data can be transformed). Where a concentration of drug kills all of the cells the effect would be '1'.

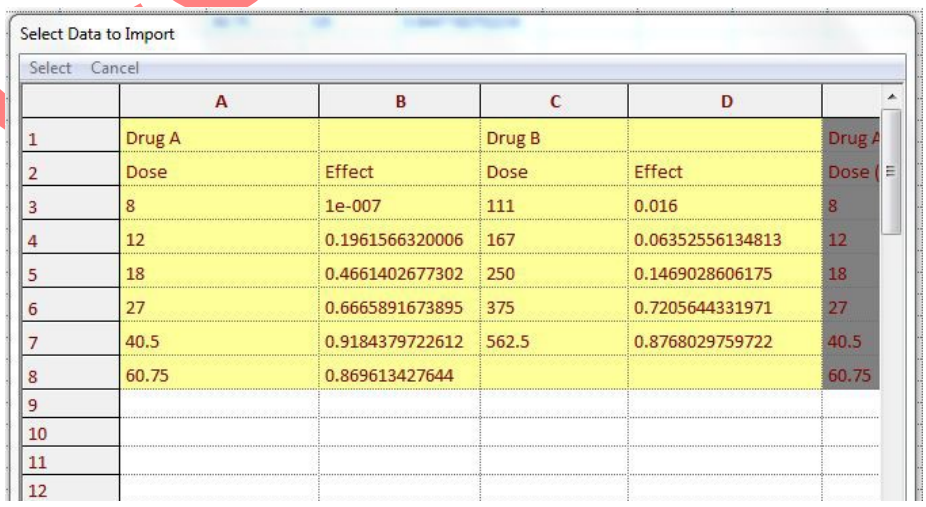
The Raw Data Grid can be found by clicking on the appropriate tab at the top of the **Contents Pane** displayed down the left hand side of the screen. If the data is not in the correct format then the results will be compromised. You cannot use the **Add Drug Wizard** if the data has not been entered correctly into the Raw Data Grid. If you wish to use the Wizard you must ensure that your original Excel data file has the data in a format identical to that shown above.

Importing raw data using the Raw Data Grid

Double click the Raw Data Grid tab in the contents pane and then select **File / Import Raw Data**' (or use the right-click menu). This will open the raw data import dialog box.

Select the **Import from File** option after which the navigation panel will open. Select either (i) Tab Delimited Text (*.txt) or (ii) Excel 97 Files (*.xls) and navigate to the file containing your data. Open the file.

If importing an Excel file, you will be presented with a dialog box that allows you to select the area of data to import as illustrated below:



	A	B	C	D	
1	Drug A		Drug B		Drug A
2	Dose	Effect	Dose	Effect	Dose (
3	8	1e-007	111	0.016	8
4	12	0.1961566320006	167	0.06352556134813	12
5	18	0.4661402677302	250	0.1469028606175	18
6	27	0.6665891673895	375	0.7205644331971	27
7	40.5	0.9184379722612	562.5	0.8768029759722	40.5
8	60.75	0.869613427644			60.75
9					
10					
11					
12					

Using your mouse, select the data to be imported and then press enter. The data will then be dropped into the raw data grid, starting in cell A1. You can only import data from the first 'sheet' of a workbook! You can only import a maximum of 50 columns and 1000 rows of data.

Text (.txt) data will be imported directly into the current cell.

Entering data for a Single Drug Using the 'Add Drug Wizard'

Select **Drugs / Add Drug Wizard** (or click the relevant toolbar button at the top of the screen). This will open the wizard. Enter the name of the drug (eg Gemcitabine), any comments you may wish to record, and the drug Units (eg.nM) in the boxes provided. Select **No** to the question 'Is this Drug a combination'. Select **Next**.

Now specify the **Dose** data you wish to use by clicking the **Select** button at the top of the active window. This will take you to the **Raw Data Grid** containing your original data - select the dose concentrations to be used. Press Return. Repeat this for the respective **Effect** data. Click the **Finish** button and the drug will be added to the experiment and shown in the Contents Pane. Clicking on the respective '**Report**' or '**Graphs**' tab in the Control Pane will reveal analysis for this particular drug. Any number of drugs can be separately added in this manner.

Entering data for a Drug Combination Using the 'Add Drug Wizard'

Entering two (or more) drugs in combination is done using the same approach as described above for single-agent drugs. Double click the Raw Data item in the Contents Pane to open the Raw Data Grid - enter data into the grid in the format shown below.

Select **Drugs / Add Drug Wizard** or click the relevant toolbar button – this will open the first page of the wizard. Enter the name of the drug (combination, mixture, Gem + Abrax, etc), any comments, and the drug units in the boxes provided. Select **Yes** for "Is this Drug a combination". Click the **Next** button.

Select the drugs that form the combination of interest from the list at the top of the box. Assuming that the drugs are in a constant ratio to each other, tick the appropriate 'Yes' box and then enter the ratio of the two drugs to each other (e.g. 100:1). Click the **Next** button. If the drugs are not in a fixed ratio to each other then tick the appropriate **No** box!

Select the **Dose** and **Effect** data for the drug combination by clicking on the respective Select buttons and highlighting the values. Click **Finish**.

Entering data for a Single Drug Using the 'Add Drug Manually'

Drug data can also be added manually without using the Drug Wizard (and without first entering the data into the Raw Data Grid).

Select **Drugs / Add Drug Manually** or click the relevant toolbar button. This will open the manual entry dialog box.

Enter the name of the drug (eg Gemcitabine), any comments you may wish to record, and the drug Units (eg.nM) in the boxes provided at the top of the dialogue window.

Enter the first pair of data points by selecting **New**. Enter the dose (e.g.0.5) and the corresponding effect (e.g.0.2). Click **OK** to complete the entry. Enter the remaining points by pressing Enter or clicking on **New** to bring up the dialogue window again. As the points are entered they will be added to the graph and once 3 or more points have been added, the Dm (median effect dose), m (the sigmoidity / shape of the curve) and r (linear correlation coefficient) values will be displayed. The graph can be changed to show the 'dose effect' or 'median effect plot' by simply selecting the appropriate button. Data points can be amended at any time by clicking on the **Edit** button. Any number of drugs can be separately added in this manner.

Entering data for a Drug Combination Using the 'Add Drug Manually'

Combination drug data can also be added manually without using the Drug Wizard (and without first entering the data into the Raw Data Grid) in the same manner as described for single-agent drugs.

Select **Drugs / Add Drug Manually** or click the relevant toolbar button. This will open the manual entry dialog box. Enter the name of the drug (eg combination), any comments you may wish to record, and the drug Units (eg.nM) in the boxes provided at the top of the dialogue window.

Select the drugs that form the combination of interest from the list at the top of the box. Assuming that the drugs are in a constant ratio to each other, tick the appropriate 'Yes' box and then enter the ratio of the two drugs to each other (e.g. 100:1; 1:250). Enter the ratio of the two drugs and then click the **Next** button.

Enter the first dataset by selecting **New**. Enter the dose (e.g.0.5) for the first drug and the corresponding effect (e.g.0.2). The dose for the second drug will be filled in for you (since you have stated that it is a fixed ratio). Click **OK** to complete the entry. Repeat the process for the remaining doses. Click **OK** and the drug combination will be added to the experiment and shown in the contents pane. Any number of combinations can be created in this way using manual entering of data.

If the drugs are not in a fixed ratio to each other then tick the appropriate **No** box! You will then be required to enter the dose data for all the drugs in your combination

using three fields (drug A dose; drug B dose; effect). You can only enter non-constant ratio drug combinations containing up to 6 drugs using the wizard. For combinations with more than 6 drugs you can create the individual drugs using the wizard but you will need to create the combination data manually.

Viewing Reports

Double click the report of interest in the contents tree – the report window will then be displayed. Text can be copied from the window and pasted into e.g. Word or Excel. The text can be printed using the File / Print command or exported using the right-click menu.

The report will include the following items (as determined by the Setup / Options in the main menu):

- experiment details – as entered by the user
- drug summary – the drug name and comment, Dm, m and r are displayed with estimated uncertainties.
- data points entered – displayed in increasing dose order.
- dose effect table – the estimated dose required to produce a given effect (within 95% confidence limits) as dictated in the Reporting Options dialog window. For combination drugs, the dose displayed is that of the first drug only.
- CI for experimental values – here is the combination index for each data point entered.
- CI simulations - the combination index for each effect value is given in this table.
- Monte Carlo simulations – mean and standard deviation values of CI using the Monte Carlo algorithm.
- DRI simulations – dose reduction index values for a range of effect values
- Summary table – a one line summary!

Viewing Graphs

Double click the Graphs of interest in the contents tree – the a selection of graphs will then be displayed. The median plot and the dose-effect plot will be presented for single drugs; combination drugs will also show a Combination Index (CI) plot.

The first time you open the summary graphs you will be asked to select the drugs to include – you can alter these later using the Select Summary Drugs (right click menu). Graphs can be printed, copied and exported in the usual manner.

5.0 Personal protection -

A Howie laboratory coat and lab gloves must be worn at all times.

6.0 Spillages -
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training - All staff should undergo training in this technique before performing the procedure.

8.0 Related documents –

8.1 'CalcuSyn for Windows – Software for Dose Effect Analysis'

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 09/09/2021

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature:  Date: 09/09/2021

QA release by:

Name: John O' Connor

Position: QA Manager

Signature:  Date: 09/09/2021

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE