


### Sample Pooling Calculator (SPoC) – worked example:

1. Enter the project name, unique Sample Donor ID and the number of samples in the appropriate fields.  
The final volume of the pooled sample is set to 1ml but this can be changed to any volume (in mls) as needed.
2. Press the “GO” button to generate a list.

Project Name	Example Project
Sample Donor ID	XX01
Number of Samples	10
Final Volume (ml)	1

GO 

Reset Form

Extend sample list

Export and Save


## SPOC

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### Instructions

- 1) Type your project name and input the sample donor ID and the number of samples you have in the input box to the left.
- 2) The final volume is set to 1ml. To change this, type a different volume (in ml) in the box.
- 3) Press "GO" to generate a table.
- 4) Enter each sample date in the white 'Date' column provided. The values in the other columns will auto-populate.
- 5) If you need to change the final volume, type in a new value in cell M5 and press Enter on your keyboard.
- 6) If you want to start over, press "Reset Form".
- 7) Once you are finished, press "Export and Save". You will be prompted to save the file with a specified name and location.
- 8) Use the calculated volumes (in  $\mu$ l) to add the correct volume of each sample to your pooled sample.

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3. Next enter the date of each sample in the fields provided.  
Note that you can increase the number of samples at any time by clicking the button “Extend sample list”.

No.	Sample Donor ID	Date	Sample Day	Unique sample Identifier	$\mu\text{L}$ to add
1	XX01	01/01/2019	0	XX01_d0	500
2	XX01	03/01/2019	2	XX01_d2	0
3	XX01	04/01/2019	0	XX01_d0	0
4	XX01		0	XX01_d0	0
5	XX01		0	XX01_d0	0
6	XX01		0	XX01_d0	0
7	XX01		0	XX01_d0	0
8	XX01		0	XX01_d0	0
9	XX01		0	XX01_d0	0
10	XX01		0	XX01_d0	0

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**Enter Date**  
Please enter the date as dd/mm/yy

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4. Once all the sample dates have been inputted, click the “Export and Save” button.


No.	Sample Donor ID	Date	Sample Day	Unique sample Identifier	$\mu\text{L}$ to add
1	XX01	01/01/2019	0	XX01_d0	71
2	XX01	03/01/2019	2	XX01_d2	107
3	XX01	04/01/2019	3	XX01_d3	71
4	XX01	05/01/2019	4	XX01_d4	71
5	XX01	06/01/2019	5	XX01_d5	107
6	XX01	08/01/2019	7	XX01_d7	143
7	XX01	10/01/2019	9	XX01_d9	107
8	XX01	11/01/2019	10	XX01_d10	71
9	XX01	12/01/2019	11	XX01_d11	143
10	XX01	15/01/2019	14	XX01_d14	107


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Sample Donor ID	XX01
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Final Volume (ml)	1

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Export and Save 

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5. The sample list opens in a new Excel workbook and the “Save As” dialogue box pops up. Save as a new excel file in the desired location.

The screenshot shows an Excel spreadsheet with the following data:

Date	Sample Day	Unique sample identifier	μL to add
01/01/2019	0	XX01_d0	71
03/01/2019	2	XX01_d2	107
04/01/2019	3	XX01_d3	71
05/01/2019	4	XX01_d4	71
06/01/2019	5	XX01_d5	107
08/01/2019	7	XX01_d7	143
10/01/2019	9	XX01_d9	107
11/01/2019	10	XX01_d10	71
12/01/2019	11	XX01_d11	143
15/01/2019	14	XX01_d14	107

Overlaid on the spreadsheet is the 'Save As' dialog box. The file name is 'Example 1', the save type is 'Excel Workbook (\*.xlsx)', and the location is 'This PC > Documents'. Metadata fields for Title, Subject, Author, and Manager are also visible.

- Column E shows the calculated volume of each sample. These volumes need to be pooled together to make up a single pooled sample. This pooled sample should be analysed in triplicate and represents an average of all the samples over the time period selected.

## Methodology

### Definitions:

1. In a study of  $n$  days ( $d$ ), the study runs from  $d_0$  to  $d_n$ . In our example above, the study runs from  $d_0$  to  $d_{14}$
2.  $s$  = sample
3.  $s_i$  is the sample number, where  $i = 1 \rightarrow z$ , so that  $s_1, s_2, s_3 \dots s_z$ , where  $i$  = the position of a value in a row vector containing the days the samples were taken ( $d_i$ ). If samples are taken less frequently than daily,  $z < n$ , and  $i$  will be discontinuous. For instance in our example above, the row vector contains the following  $d_i$  values: 0, 2, 3, 4, 5, 7, 9, 10, 11, 14. In our example above,  $z$  is 10,  $n=14$ .
4.  $V$  is the final volume of the pooled sample. In our example above,  $V=1\text{ml}$
5.  $v_i$  is the volume that sample  $s_i$  will contribute to  $V$ , so that  $V = v_1 + v_2 + v_3 \dots v_z$ . In our example above,  $V = 71 + 107 + 71 + 71 + 107 + 143 + 107 + 71 + 143 + 107\mu\text{l} = 1\text{ml}$

### Algorithm:

The aim is to replace analysis of multiple individual samples with analysis of a single pooled sample. Hence the relative volume contribution of each individual sample to the single pooled sample needs to be proportional to the time interval between the individual sample and its neighbours.

1. Each sample was assumed to reflect the UNCR of:
  - a) the day it was taken, i.e.  $d_i$
  - b) half the inter-sample time interval preceding day  $i$ , i.e.  $d_i - d_{(i-1)}$
  - c) half the inter-sample time interval following day  $i$ , i.e.  $d_{(i+1)} - d_i$

Hence the UNCR of sample  $s_i$  is assumed to reflect the UNCR of the time period surrounding it, i.e.

$$t_i = \frac{d_{(i+1 \rightarrow z)} - d_{(i-1)}}{2}$$

See figure across. For example, for sample  $s_0$ ,  $v_0 = (2-0)/2 = 1$  day; for sample  $s_5$ ,  $v_5 = (7-4)/2 = 1.5$  days, for sample  $s_{10}$ ,  $v_{10} = (14-11)/2 = 1.5$  days.

2. The contribution of sample  $s_i$  to  $V$ , i.e.  $v_i$ , is this time period  $t_i$  divided by the total time period ( $t_n$  in days), multiplied by  $V$  in  $\mu\text{l}$ , so

$$v_i = \frac{t_i}{n} / (1000 \times V)$$

