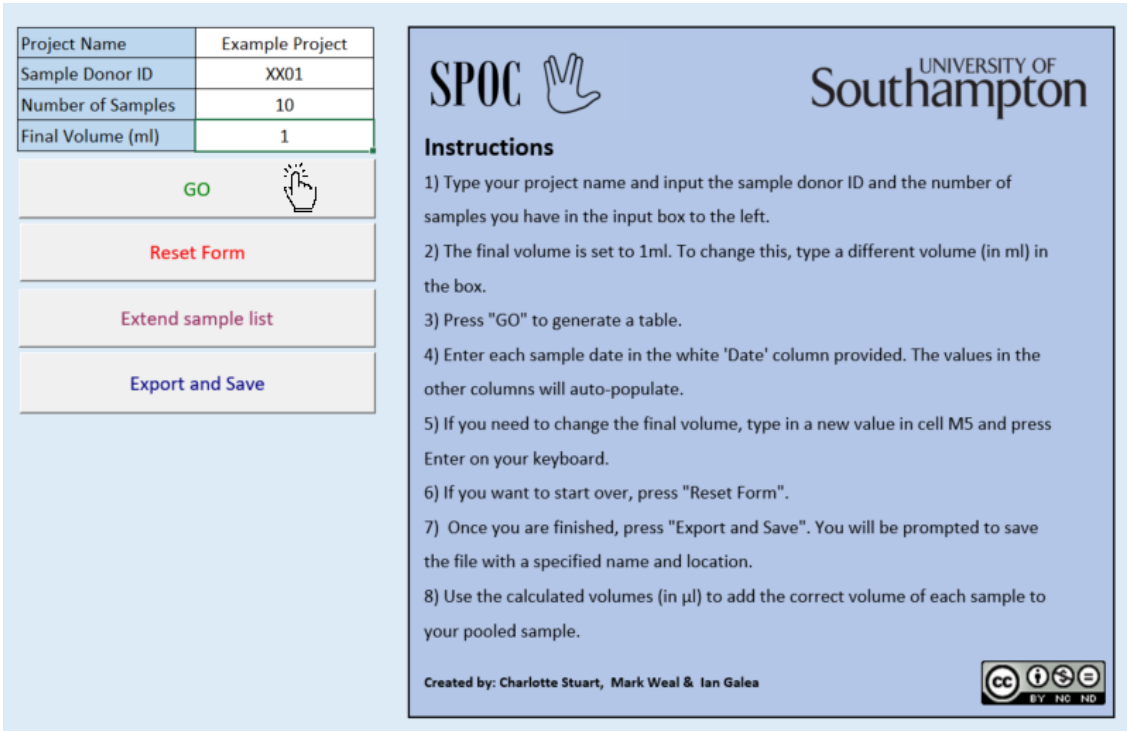
Sample Pooling Calculator (SPoC) – worked example:

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.



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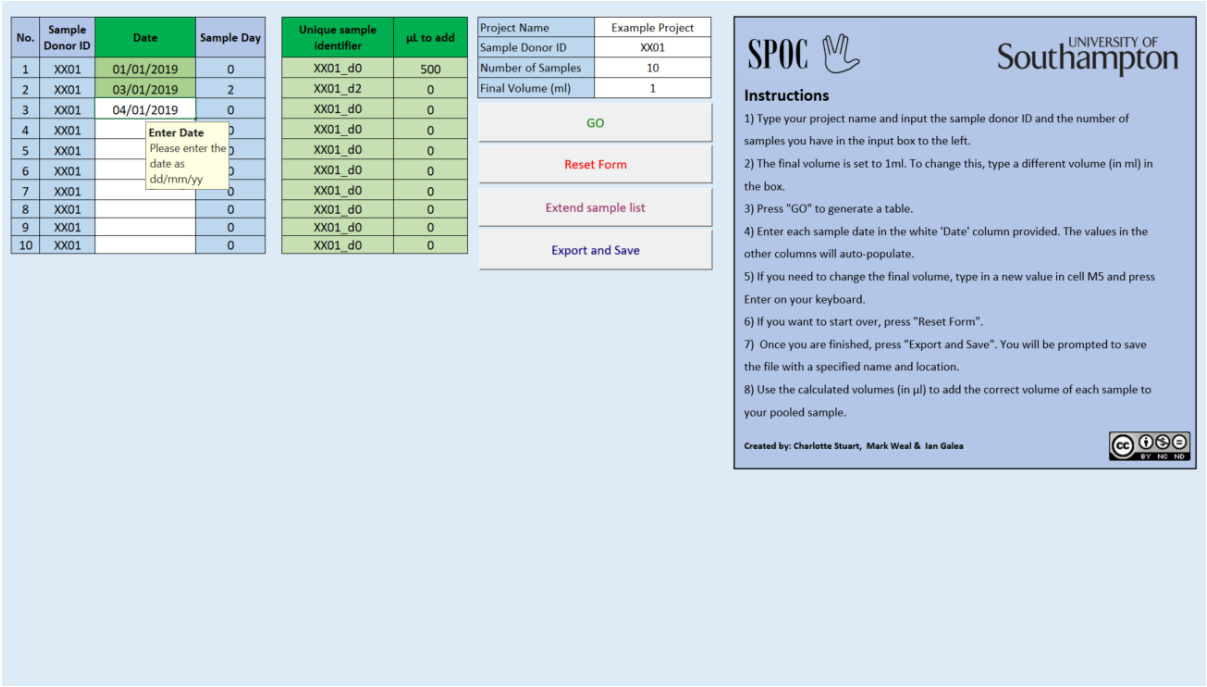
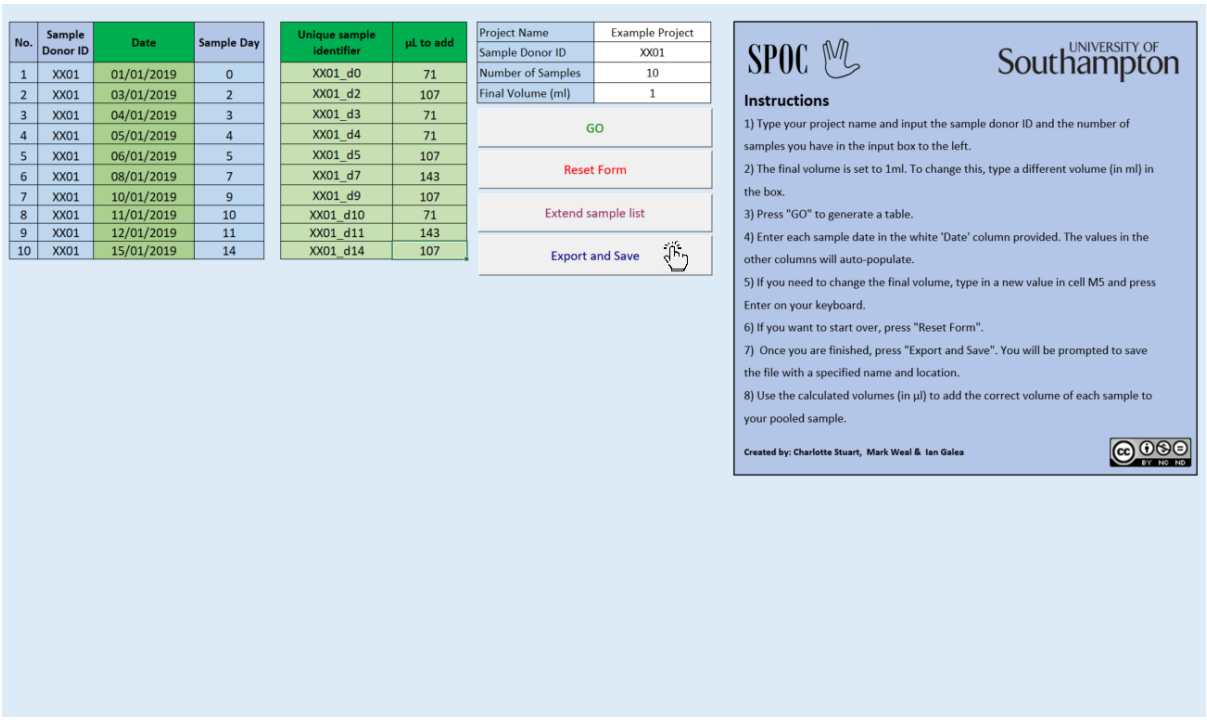
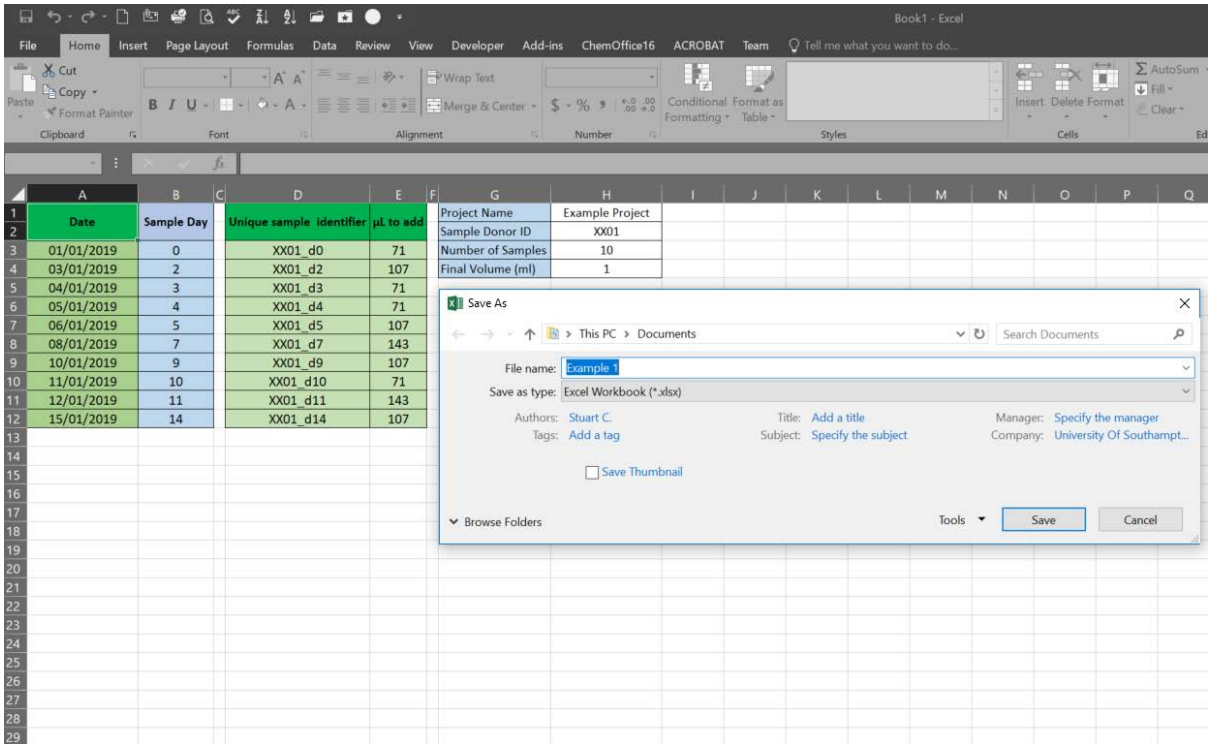
3. Next enter the date of each sample in the fields provided.
   Note that you can increase the number of samples at any time by clicking the button "Extend sample list".



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



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.



6. 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.

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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 ….. 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=1ml
5. v_i is the volume that sample s_i will contribute to V, so that V = v_1 + v_2 + v_3 …… v_z. In our example above, V = 71 + 107 + 71 + 71 + 107 + 143 + 107 + 71 + 143 + 107µl = 1ml

Algorithm:

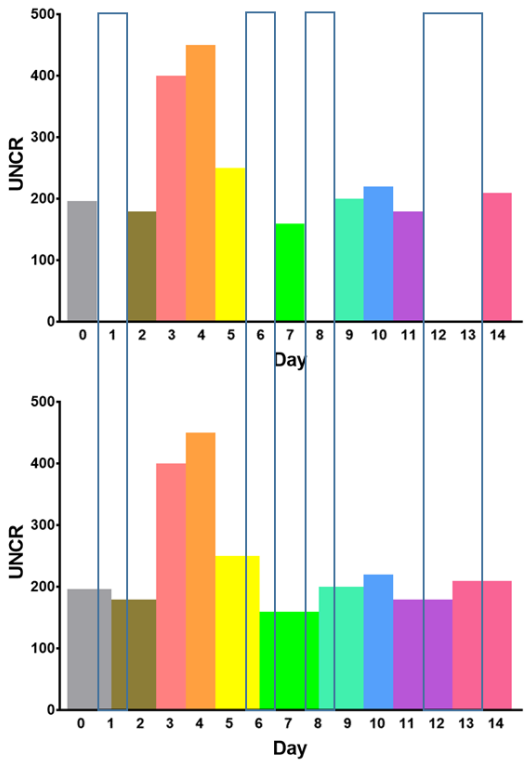
The aim is to replace analysis of multiple individual samples with analysis of a single pooled sample. Hence the relative volume contribution of each individual sample to the single pooled sample needs to be proportional to the time interval between the individual sample and its neighbours.

1. Each sample was assumed to reflect the UNCR of:
   a) the day it was taken, ie 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 µl, so

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

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