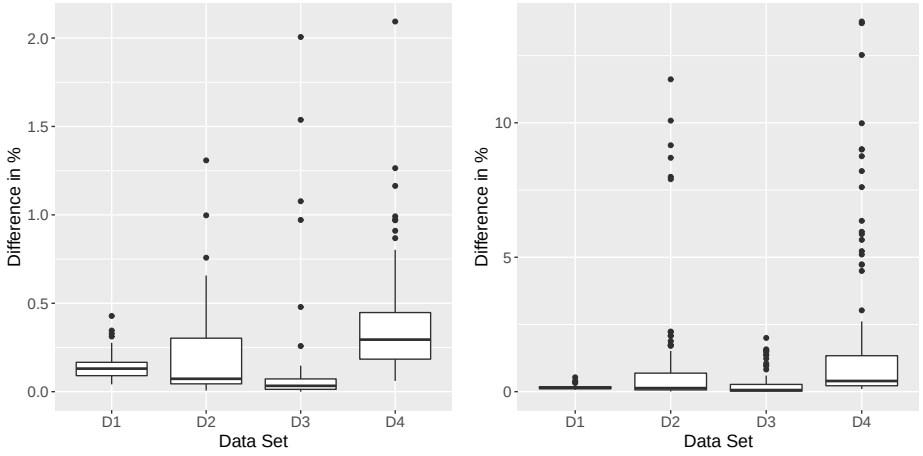
We present the results of *ddPCRclust* for four ddPCR datasets. Dataset D1 comprises 72 reactions, the others comprise 96 reactions each. For each reaction r, we calculate the difference d_r according to Equation 1,

$$d_r = \frac{\sum_{i=1}^{t} |auto_i - man_i|}{total_r} \cdot 100 \tag{1}$$

where t is the number of targets in this reaction, $auto_i$ the number of positive droplets for target i according to the *ddPCRclust* algorithm, man_i the number of positive droplets for target i according to manual annotation and $total_r$ the total number of droplets in this reaction.

The mean difference over all 360 reaction between *ddPCRclust* and manual analysis is $\sim 0.21\,\%$, being as low as $\sim 0.10\,\%$ for D3, while performing worst for D4 with a mean difference of $\sim 0.37\,\%$. It becomes evident that the accuracy of the algorithm depends on the compactness of the clusters. The run time underlines this fact: D4 suffered from a low amplification efficiency, being more than twice as fast as the other datasets, while showing the highest difference.



(a) Automatic clustering vs manual

(b) Fast mode vs manual

Figure S1: The difference between automatic clustering and manual analysis for selected datasets. In (a) the results for the full algorithm are shown, while (b) presents the results for the fast mode.

Table S1: Run time of ddPCRcust computed on Intel(R) Core(TM) i7-4650U CPU @ 1.70GHz and 8 GB RAM for selected datasets.

	D1	D2	D3	D4
Number of reactions	72	96	96	96
Fast mode run time	67 s	17 s	13 s	18 s
Full mode run time	333 s	309 s	360 s	144 s