Effect of some essential oils on phagocytosis and complement system activity

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Supplementary data

Examples of histograms and data obtained from flow cytometry in the experiments on phagocytic activity by human neutrophils

Two examples of histograms and data obtained from flow cytometry in the experiments on phagocytic activity by human neutrophils are included in the present document. The first one corresponds to nutmeg terpenes, showing inhibitory activity at the highest concentrations, and the second one corresponds to the mixture of bornyl and isobornyl acetates, which showed stimulant activity at the highest concentration.

Each example corresponds to one experiment out of the four included in the statistical analysis. For each example, the histograms for the **control** (neutrophils without any treatment), **neutroplhils + LPS** (neutrophils stimulated with lipopolysaccharides -LPS-, which was used as positive control), and the neutrophils treated with different concentrations of the **tested substance** are shown, together with a table of the results on the percentage of viable neutrophils that phagocyted one or more fluorescent particles in relation to the total number of viable neutrophils.

The different regions of the neutrophil population considered are indicated by R and a number. Those regions correspond to:
R3: Viable neutrophils having phagocyted at least one fluorescent particle.
R5: Viable neutrophils having phagocyted one fluorescent particle.
R8: Viable neutrophils having phagocyted two fluorescent particles.
R9: Viable neutrophils having phagocyted three fluorescent particles.
R10: Viable neutrophils having phagocyted four fluorescent particles.

Histogram and data are also given for **non-specific adhesion control**. This control measures the proportion of fluorescent particles adhered to the cell but non phagocyted. Obviously, in this case regions R3, R5, R8, R9 and R10 correspond to neutrophils having a number of fluorescent particles adhered to the cell surface, but not phagocyted.

Results on phagocytosis presented in the paper were calculated from the data corresponding to R3, measuring the viable neutrophils having phagocyted at least one fluorescent particle.

Example 1: nutmeg terpenes

Figure 1 shows the histograms obtained from one experiment for nutmeg terpenes, and **Table 1** shows the percentage of viable neutrophils found in each region of the neutrophil population considered.

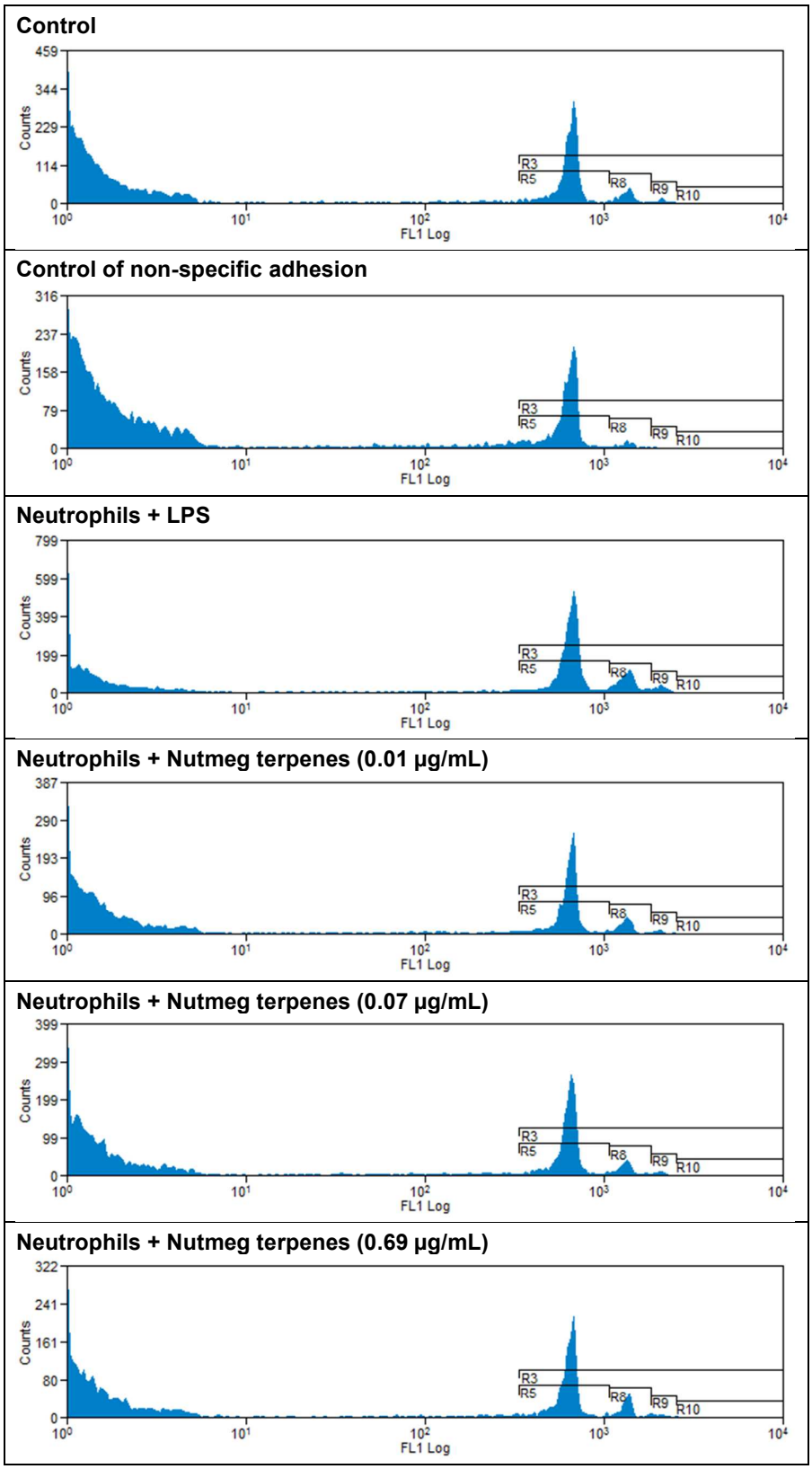

Figure 1. Histograms of a representative experiment studying the effect of nutmeg terpenes on phagocytic activity of neutrophils.

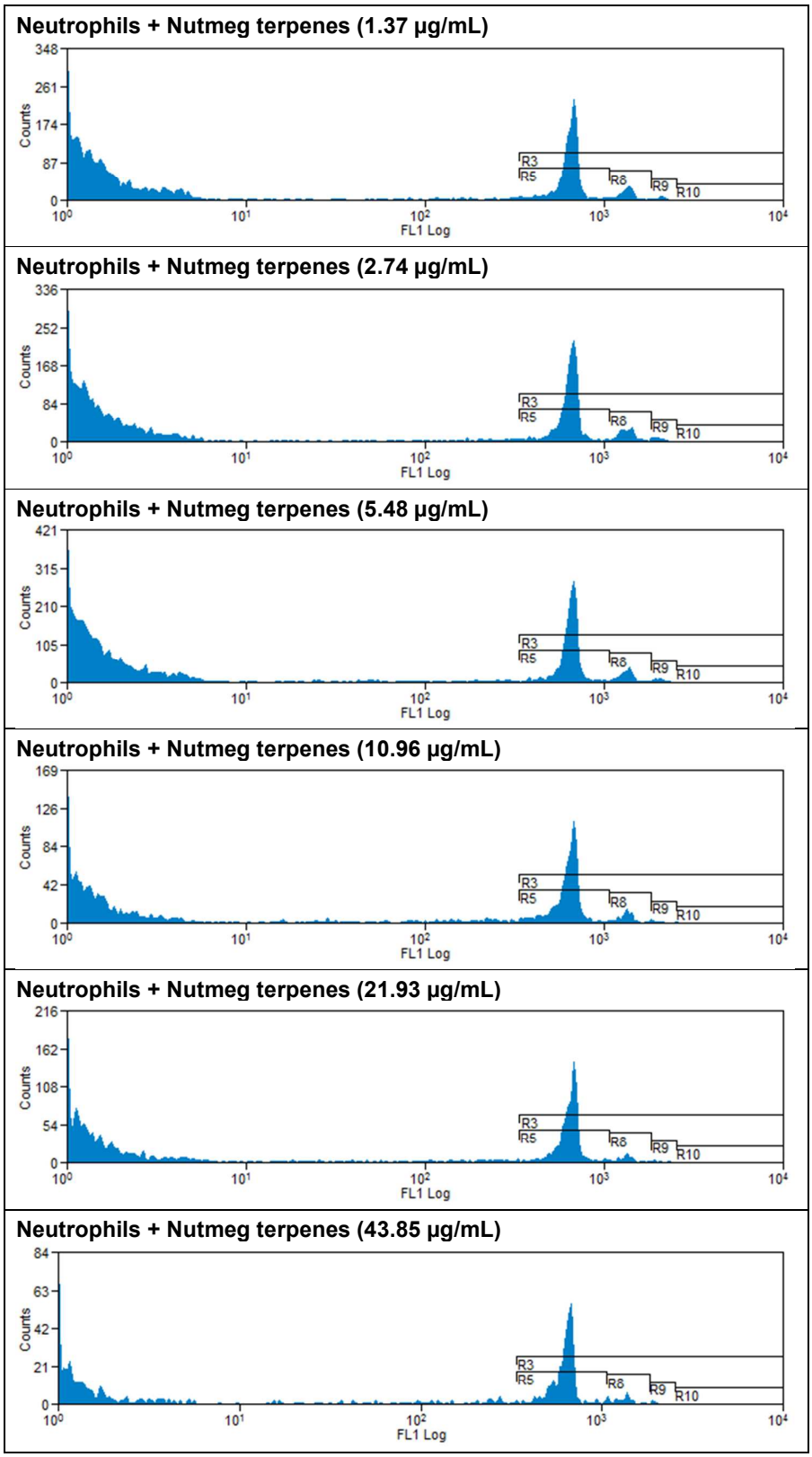

Figure 1 (Cont.). Histograms of a representative experiment studying the effect of nutmeg terpenes on phagocytic activity of neutrophils.

Table 1. Percentage of viable neutrophils phagociting one or more fluorescent particles in one representative experiment in which nutmeg terpenes were tested.

Region	Control of non-specific adhesion	Control	LPS	Nutmeg terpenes (µg/mL)								
				0.01	0.07	0.69	1.37	2.74	5.48	10.96	21.93	43.85
R3	4.41	9.47	19.30	10.34	10.32	9.94	9.58	9.62	9.72	7.91	7.69	7.24
R5	4.14	8.06	14.93	8.60	8.81	8.20	8.20	8.12	8.23	7.01	7.03	6.56
R8	0.25	1.21	3.61	1.45	1.29	1.55	1.18	1.29	1.23	0.79	0.61	0.66
R9	0.02	0.26	0.90	0.31	0.29	0.28	0.24	0.25	0.29	0.11	0.10	0.07
R10	0.00	0.00	0.04	0.03	0.01	0.03	0.00	0.00	0.01	0.02	0.00	0.00

Example 2: Mixture of bornyl and isobornyl acetates

Figure 2 shows the histograms obtained from one experiment for the mixture of bornyl and isobornyl acetates, and **Table 1** shows the percentage of viable neutrophils found in each region of the neutrophil population considered. The mixture tested contained 76.8% of bornyl acetate and 21.7% of isobornyl acetate.

Table 2. Percentage of viable neutrophils phagociting one or more fluorescent particles in one representative experiment in which the mixture of bornyl and isobornyl acetates were tested.

Region	Control of non-specific adhesion	Control	LPS	Bornyl acetate + isobornyl acetate (µg/mL)								
				0.01	0.09	0.87	1.75	3.49	6.99	13.98	27.95	55.90
R3	1.56	6.75	22.70	6.76	7.31	7.42	7.26	7.18	7.32	7.19	7.46	8.32
R5	1.44	5.68	15.61	5.73	6.17	6.23	6.15	6.10	6.08	6.07	6.28	7.35
R8	0.10	0.91	5.19	0.87	1.01	1.02	0.95	0.93	1.07	0.90	1.05	0.91
R9	0.02	0.18	2.21	0.18	0.17	0.20	0.19	0.17	0.23	0.26	0.17	0.10
R10	0.00	0.01	0.09	0.00	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.01

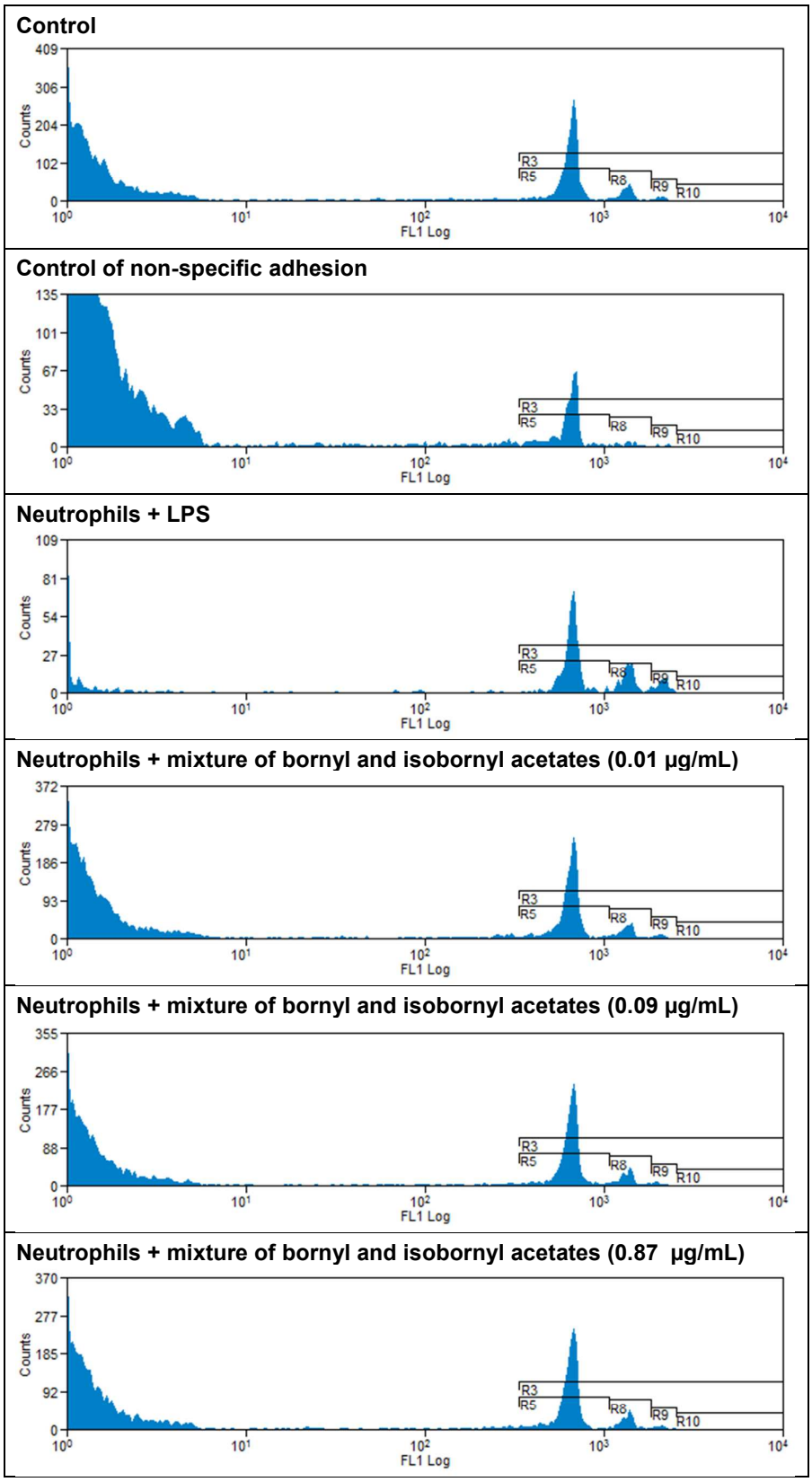

Figure 2. Histograms of a representative experiment studying the effect of the mixture of bornyl and isobornyl acetates on phagocytic activity of neutrophils.

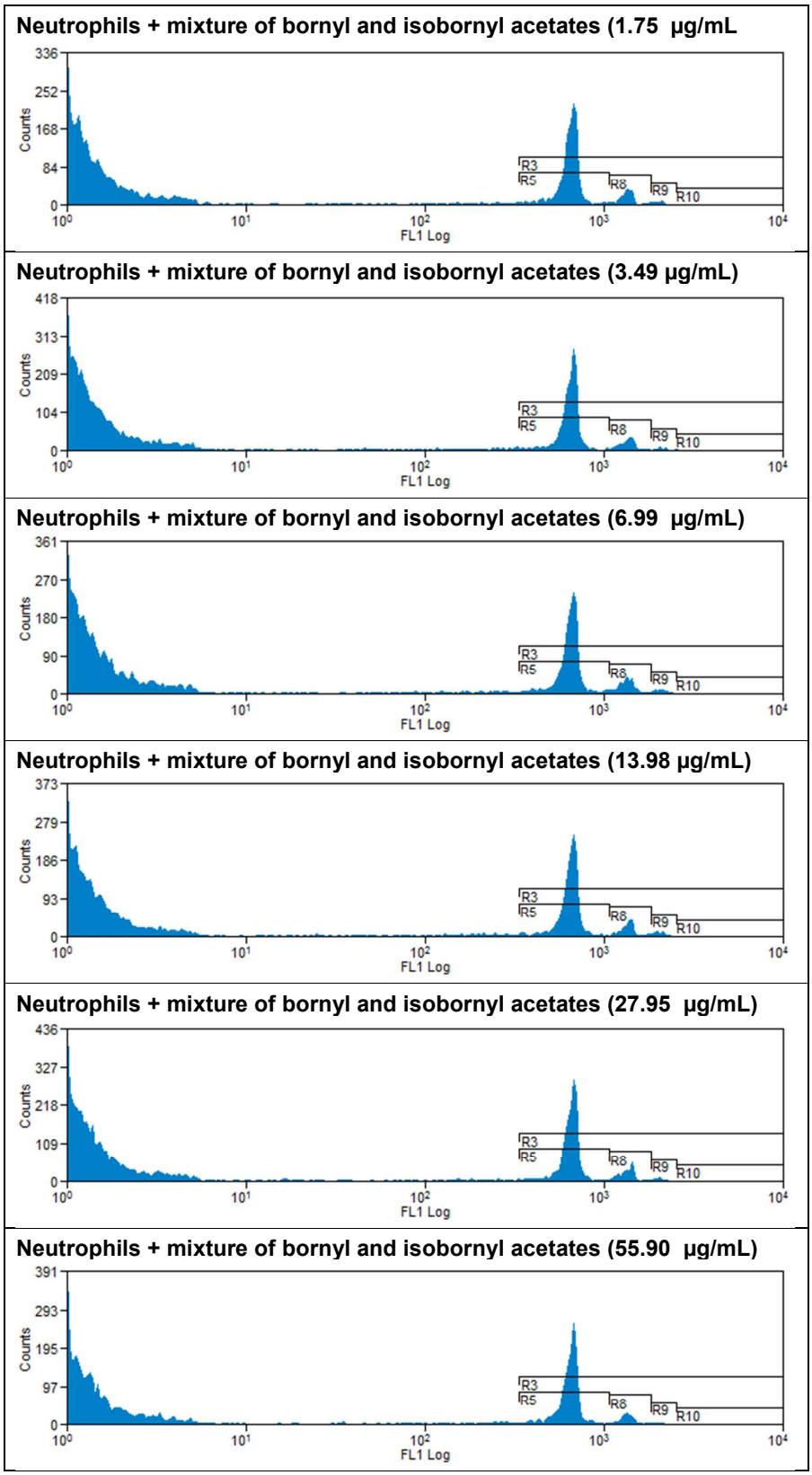

Figure 2 (Cont.). Histograms of a representative experiment studying the effect of the mixture of bornyl and isobornyl acetates on phagocytic activity of neutrophils.