

Flow cytometric analysis of mechanically dissociated blood clot obtained from serum separator tubes

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Background

Flow cytometry is a versatile powerful technology based on analysis of cellular suspensions, obtained by whole blood samples collected in anticoagulant tubes or by mechanical dissociation of matrices and solid tissues. In the human routine clinical practice, the use of serum blood collection tubes for flow cytometry assays is not very usual. In livestock, each animal is regularly subjected to serological tests in accordance with national and/or regional prophylaxis plans. These tests are performed using only serum blood collection tubes that however may be used for additional flow cytometry tests.

Aim of Study

Aim of this is the flow cytometric evaluation of the recovered leukocytes by mechanical clot dissociation using MEDIMAX apparatus.



Methods

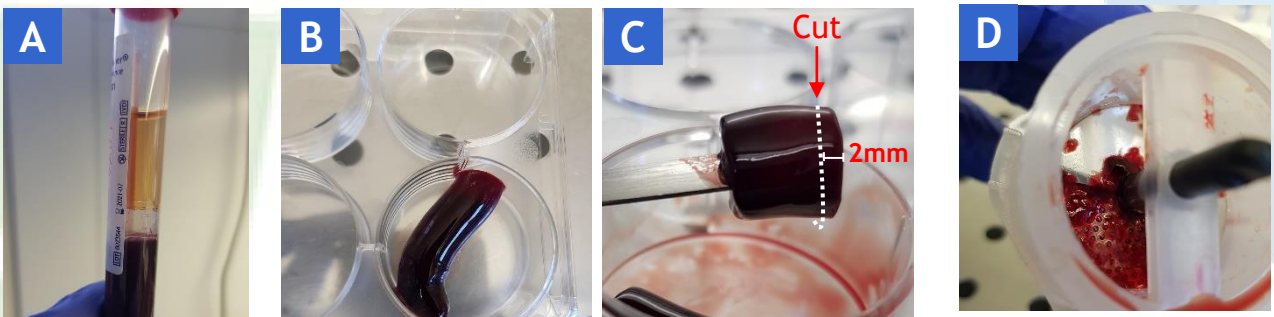
The serum of a cow was collected in three serum separator tubes (BD SSTTM II Advance).

After centrifugation (1300g for 10 min) the serum of each tube (Fig. 1A) was removed and gel separator was discarded.

The clots were recovered by inversion on a 6-well cell culture plate (Fig. 1B). A disc (about 2 mm thick, Fig. 1C) was cut and chopped with a scalpel. It was inserted into a Medicon Max with 15mL of cold PBS, and was disaggregated using MEDIMAX apparatus (CTSV, Bruino-TO, Italy) for 30 sec (Fig. 1D). The cellular suspension was recovered by a syringe through the syringe port on the Medicon Max and filtered through a 30- μ m Filcon into 15mL sterile tube (Fig. 1E).

After centrifugation, the cellular pellet was lysed by 15mL of 1X RBC Lysis Buffer Multi-species (ThermoFisher) for 10 min. The recovered leukocytes were labelled with a 6-color panel for T lymphocytes characterization.

A heparinized whole blood sample was used as control.

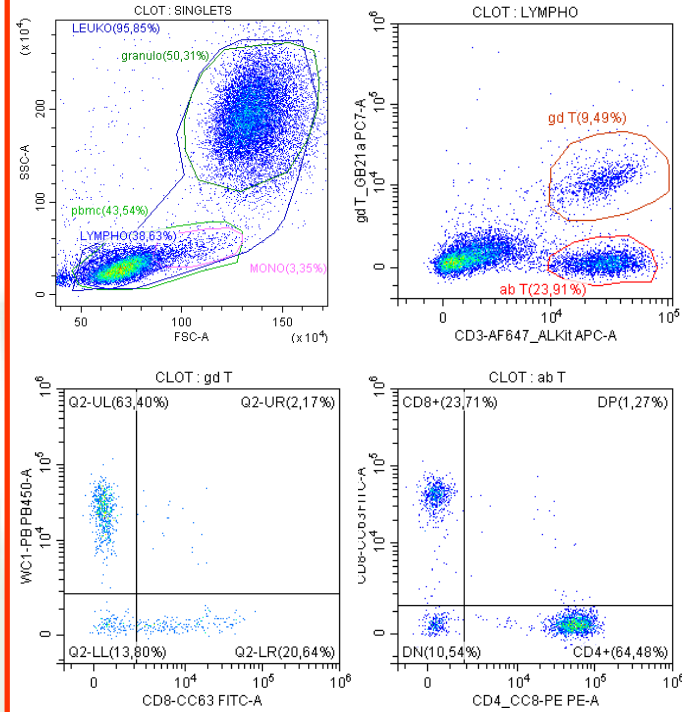


Results and Conclusions

No significant differences were found between the percentages of CD4⁺, CD8⁺, $\gamma\delta$, and WC1 T lymphocytes obtained from clot or heparinized whole blood samples. Our preliminary results showed that the MEDIMAX apparatus can be validly used to recover lymphocytes from clots of human and veterinary samples



Clot

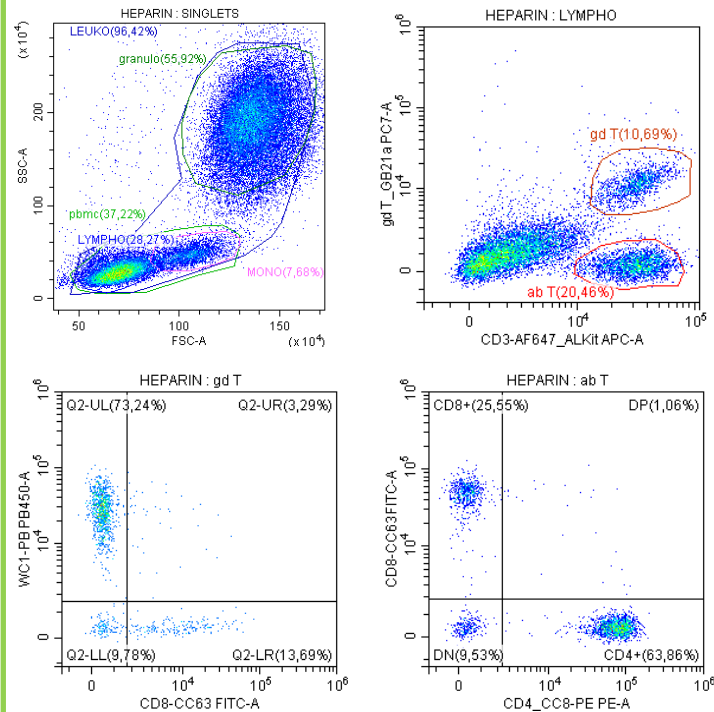


Tube Name: CLOT

Sample ID:

Population	Events	% Total	% Par...
● All Events	26176	100,00%	100,0...
● TIME	25913	99,00%	99,00%
● SINGLETS	23927	91,41%	91,41%
● MONO	801	3,06%	3,35%
● LEUKO	22933	87,61%	95,85%
● LYMPHO	9243	35,31%	38,63%
> ● CD3+	3152	12,04%	34,10%
> ● gd T	877	3,35%	9,49%
● ab T	2210	8,44%	23,91%
⊗ DP	28	0,11%	1,27%
⊗ CD8+	524	2,00%	23,71%
⊗ DN	233	0,89%	10,54%
⊗ CD4+	1425	5,44%	64,48%
● pbmc	10417	39,80%	43,54%
● granulo	12037	45,98%	50,31%
● Neutrophils	10375	39,64%	86,19%
● Eosinophils	1451	5,54%	12,05%

Heparin



Tube Name: HEPARIN

Sample ID:

Population	Events	% Total	% Par...
● All Events	44911	100,00%	100,0...
● TIME	44748	99,64%	99,64%
● SINGLETS	42290	94,16%	94,16%
● MONO	3246	7,23%	7,68%
● LEUKO	40774	90,79%	96,42%
● LYMPHO	11957	26,62%	28,27%
> ● CD3+	3596	8,01%	30,07%
> ● gd T	1278	2,85%	10,69%
● ab T	2446	5,45%	20,46%
⊗ DP	26	0,06%	1,06%
⊗ CD8+	625	1,39%	25,55%
⊗ DN	233	0,52%	9,53%
⊗ CD4+	1562	3,48%	63,86%
● pbmc	15739	35,04%	37,22%
● granulo	23648	52,66%	55,92%
● Neutrophils	19758	43,99%	83,55%
● Eosinophils	3459	7,70%	14,63%