

This is the peer reviewed version of the following article:

Comazzi S, Avery PR, Garden OA, Riondato F, Rütgen B, Vernau W and Vernau W.  
European Canine Lymphoma Network Consensus Recommendations for Reporting Flow  
Cytometry in Canine Hematopoietic Neoplasms. Cytometry Part B 2016:

[10.1002/cyto.b.21382](https://doi.org/10.1002/cyto.b.21382)

This article may be used for non-commercial purposes in accordance with [Wiley Terms and Conditions for Self-Archiving](#).

The full details of the published version of the article are as follows:

TITLE: European canine lymphoma network consensus recommendations for reporting flow  
cytometry in canine hematopoietic neoplasms

AUTHORS: S. Comazzi, P. R. Avery, O. A. Garden, F. Riondato, B. Rütgen, W. Vernau, on  
behalf of the European Canine Lymphoma Network

JOURNAL TITLE: Cytometry Part B

PUBLISHER: Wiley

PUBLICATION DATE: 28 June 2016 (online)

DOI: 10.1002/cyto.b.21382

1 Regional spotlight

2

3 EUROPEAN CANINE LYMPHOMA NETWORK CONSENSUS RECOMMENDATIONS FOR REPORTING FLOW  
4 CYTOMETRY IN CANINE HEMATOPOIETIC NEOPLASMS

5

6 Comazzi S.<sup>1</sup>, Avery PR<sup>2</sup>, Garden OA<sup>3</sup>, Riondato F<sup>4</sup>, Rütgen B<sup>5</sup>, Vernau W<sup>6</sup>, on behalf of the European Canine  
7 Lymphoma Network

8

9

10

Running title: reporting flow cytometry in canine lymphoma

11

<sup>1</sup> Department of Veterinary Science and Public Health, University of Milan, Milan, IT,

12

<sup>2</sup> Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, US,

13

<sup>3</sup> Immune Regulation Laboratory, Department of Clinical Science and Services, Royal Veterinary College, London, UK

14

<sup>4</sup> Department of Veterinary Sciences, University of Turin, Turin, IT,

15

<sup>5</sup> Clinical Pathology, Department of Pathobiology, University of Veterinary Medicine Vienna, AT,

16

<sup>6</sup> Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California-  
18 Davis, Davis, CA,US,

17

18

19

Corresponding author:

20

Stefano Comazzi

21

Department of Veterinary Science and Public Health,

22

University of Milan, Italy,

23

e-mail: stefano.comazzi@unimi.it, stefano.comazzi@eu-can-lymph.net,

24

tel ++39 02 503 18053, Fax ++39 02 503 18095

25

26

Keywords, dog, lymphoma, European Canine Lymphoma Network, report, guidelines.

27

28

29

30

31

32

33 Abstract

34

35 Background: Flow cytometry (FC) is assuming increasing importance in diagnosis in veterinary oncology. The  
36 European Canine Lymphoma Network (ECLN) is an international cooperation of different institutions working on  
37 canine lymphoma diagnosis and therapy. The ECLN panel of experts on FC has defined the issue of reporting FC on  
38 canine lymphoma and leukemia as their first hot topic, since a standardized report that includes all the important  
39 information is still lacking in veterinary medicine.

40

41 Methods: The flow cytometry panel of the ECLN started a consensus initiative using the Delphi approach. Clinicians  
42 were considered the main target of FC reports. A panel of experts in FC was interrogated about the important  
43 information needed from a report.

44

45 Results: Using the feedback from clinicians and subsequent discussion, a list of information to be included in the report  
46 was made, with four different levels of recommendation. The final report should include both a quantitative part and a  
47 qualitative or descriptive part with interpretation of the salient results. Other items discussed included the necessity of  
48 reporting data regarding the quality of samples, use of absolute numbers of positive cells, cutoff values, the intensity of  
49 fluorescence, and possible aberrant patterns of antigen expression useful from a clinical point of view.

50

51 Conclusion: The consensus initiative is a first step towards standardization of diagnostic approach to canine  
52 hematopoietic neoplasms among different institutions and countries. This harmonization will improve communication  
53 and patient care and also facilitate the multicenter studies necessary to further our knowledge of canine hematopoietic  
54 neoplasms.

55

56 Flow cytometry (FC) is increasingly being used in veterinary clinical pathology laboratories owing to the increasing  
57 number of FC facilities, greater availability of specific antibodies labeled with different fluorochromes, and the rapidity  
58 with which results can be generated. Immunophenotyping hematopoietic neoplasms is one of the most important  
59 applications of FC in veterinary clinical pathology diagnostics since this method rapidly provides useful information on  
60 the lineage of neoplastic cells, identifies some specific neoplastic subtypes (T zone lymphomas, chronic lymphocytic  
61 leukemia) [1-4], accurately defines stage [5] and, in some cases, can detect minimal residual disease [6].  
62

63 The overall utility of flow cytometry and its role in the diagnostic pathway is dependent on several aspects. One aspect  
64 is the ability to provide important information to frame the neoplastic disease in the context of results of other clinical  
65 and laboratory tests. Therefore, a useful FC report should contain all the appropriate information required by clinicians,  
66 provide improved characterization of the neoplastic disease, help determine therapy, and inform a monitoring strategy  
67 to enable early detection of relapse. This requires sufficient clarity of reporting to allow understanding by non-FC  
68 experts. The specific experience of the flow cytometrist should help in the interpretation of the results, always in  
69 conjunction with signalment, clinical presentation, and the results of other laboratory tests. However, an appropriate  
70 report should also contain all necessary raw data and describe the strategies used to generate them. This provides  
71 information about reproducibility and facilitates interpretation by other experts in second opinion or multicenter  
72 studies. The balance between these two aspects, the clinician-friendly utility and the inclusion of sufficient technical  
73 information to represent a rigorous and reproducible analysis, shapes the format of the FC report.  
74

75 In human medicine, the issue of reporting flow cytometry immunophenotyping has been widely debated [7, 8] and  
76 guidelines have been published and regularly updated [9, 10]. Until now, there has been no similar discussion in  
77 veterinary medicine. To the authors' knowledge, every veterinary FC facility uses its own strategy and system in  
78 reporting results of immunophenotyping.  
79

## 80 **Methods**

81  
82 The European Canine Lymphoma Network (ECLN) is a network that was created in 2009 with the aim of establishing  
83 cooperation among different institutions working on canine lymphoma diagnosis and therapy [11]. The definition of  
84 consensus guidelines and approaches is one of the main goals of ECLN and a specific panel on FC has been formed  
85 gathering 16 participants selected on demonstrated expertise in veterinary FC. The ECLN aims to drive the creation of  
86 consensus guidelines, possibly interfacing with extra EU specialists, in a democratic and inclusive way. The FC panel of  
87 the ECLN defined the issue of reporting FC immunophenotyping as the first "hot" topic requiring a consensus  
88 discussion.  
89

90 When attempting to reach a consensus on defined topics using online-based surveys, a tool called the Delphi method is  
91 considered the best technique [12]. This method relies on a series of questionnaires provided to the panel participants  
92 in "rounds" until answers converge towards a common answer. Only statements reaching at least 75% consensus  
93 agreement are included in the final document, otherwise statements are re-written and additional rounds of questions  
94 follow until the threshold agreement level of 75% is reached. After review of the results of the explorative survey and  
95 the relevant reference sources, the members of the FC panel are approached through successive rounds of questioning  
96 and the answers and feedback are collected in an anonymous fashion. The statements passing the threshold are finally  
97 considered as common recommendations and contribute to the consensus paper.  
98

99 The panel of experts in flow cytometry of the ECLN was also interrogated for each piece of information with four  
100 different levels of recommendation: mandatory, recommended, additional, or irrelevant. Results on the percentage of  
101 agreement are reported in Table 1 (16/16 responders). Relevance of information was based on a 75% response  
102 threshold. If >75% of respondents answered "mandatory", "recommended," or "additional" for a specific category  
103 without either response necessarily reaching 75% individually, the category was considered "relevant." The consensus  
104 level of recommendation was reported if it reached at least 50% of agreement among responders. If agreement was  
105 <50% for any level, the final recommendation was reported as "no consensus."  
106

107 Before starting with the discussion on different issues, the panel of experts in FC decided to document the perceived

108 needs of clinicians. A preliminary exploratory survey among the members of the ECLN therapy working group,  
109 consisting of clinicians interested in the study and therapy of canine lymphoma, was conducted. The complete results  
110 obtained from this survey are not included in detail in the present document but were used as a starting draft for the  
111 members of the FC panel for discussion in order to define the relevance of each component to be submitted to this  
112 consensus evaluation.

113

## 114 **Results**

115

### 116 *Preliminary Exploratory Survey Among the Members of the ECLN Therapy Working Group*

117

118 Thirty out of 66 clinicians (45%) of the ECLN therapy working group responded to the preliminary survey. Most (51.7%)  
119 respondents reported requesting FC in >80% of lymphoma cases, and 17.2% of respondents reported requesting FC in  
120 >50% but <80% of lymphoma cases. Only 24.1% of respondents reported the use of FC in <20% of lymphoma cases.  
121 Most respondents (51.7%) also reported sending blood and bone marrow samples for staging in selected cases, while  
122 63.3% stated that they usually add smears from blood, bone marrow or lymph node for concurrent cytomorphologic  
123 evaluation.

124

125 The majority of clinicians (58.6%) reported the definition of the immunophenotype of the neoplasm as the main reason  
126 for requiring FC, while refining lymphoma subtype (20.7%), definition of prognosis (6.9%), checking minimal residual  
127 disease (3.4%) and differentiating lymphoma from reactive conditions (3.4%) were reasons reported less frequently.

128

129 The minimally invasive nature of sampling (40%), the accuracy in resolving reactive vs. neoplastic conditions (16%) and  
130 the rapidity of the results (12%) were recognized as the main advantages of FC over other techniques. The most  
131 important mentioned characteristics required for a flow cytometric report were that they should first be both  
132 exhaustive and accurate (46.4%) and second, easy to read and interpret (42.9%).

133

134 Results were variable among respondents, indicating that the needs of clinicians are quite heterogeneous. However, the  
135 following components were considered to be essential information, with a consensus of >75% of responders:  
136 specification of the type of tissue sent, assessment of the quality of the sample, reliability of documented marker  
137 expression dependent on sample quality, panel of antibodies applied and aberrant patterns identified. All the other  
138 information (the same detailed in Table 1) was generally considered as important or essential (although without 75%  
139 consensus), except specification of the instrument used for analysis, which was considered irrelevant information by  
140 most clinicians.

141

### 142 *Identification of Discussion Topics*

143

144 Two major and eight minor issues were identified and subsequently discussed, the discussion including published  
145 sources both in human and veterinary medicine.

146

## 147 **Major issues**

148

### 149 **Identification of the “target users and/or recipients” of FC reports and their needs**

150

151 The identification of the final “targets” of FC immunophenotyping reporting on canine lymphomas and leukemias is  
152 crucial for appropriately defining the relevant information to be included in the report.

153

154 Three main “target user and/or recipient groups” with different needs were identified: (1) owners, interested in  
155 understanding their pet's disease; (2) clinicians, mainly specialists in oncology, interested in characterizing the disease in  
156 order to define prognosis, target therapy and monitor follow-up; and (3) clinical pathologists, sometimes specialists in  
157 FC, asked to interpret the results of laboratory tests in an integrated way or to provide a second opinion. The relevance  
158 and emphasis of each piece of information provided in the FC report is variable among these three user groups.

159

160 Clinicians were considered the most important target users of FC reports as they decide if the FC analysis is indicated,  
161 provide the samples, communicate the results to owners and institute treatment.

162  
163 Pet owners were not considered the major target of FC reports since management by the clinician/oncologist is the  
164 most common situation. They are generally interested in an accurate diagnosis of the disease from which their pet is  
165 suffering. Bibliographic references to support the diagnosis and better clarify the disease biology should be considered  
166 optional items in generating a final report. Mentioning the operator/specialist's name and titles should be included as  
167 well.

168  
169 Finally, other specialists such as clinical pathologists and flow cytometrists were considered possible targets of FC  
170 reports. Their needs are generally much more related to technical aspects, such as data on gating strategies, possible  
171 artefactual changes, viability/conservation of the sample, raw percentages (and absolute numbers) of neoplastic cells,  
172 type of labeling used (multicolor vs. monocolour; composition of tubes, antibody clones used), and controls. Possibly  
173 scattergrams could be used to better clarify some of the technical aspects. However, the inclusion of such technical  
174 information in the standard FC report could appear extraneous and lead to confusion of non-experts in FC. This  
175 information should be omitted from the standard FC report but could remain available to be provided upon request,  
176 including, if necessary, raw FCS files.

### 177 178 **Reporting percentages vs. descriptive report**

179  
180 Percentages and possibly absolute numbers commonly form the basis for FC immunophenotyping. This method is  
181 considered more objective and accurate; however, a series of problems may be encountered: (1) percentages are  
182 directly dependent upon gating strategy (scatter properties vs. CD45 positive cells vs. specific subtypes), (2) percentages  
183 of positive events do not provide any information regarding co-expression, (3) percentage of positivity is highly  
184 dependent on controls used to set cutoff values, (4) providing percentage of positive events alone often cannot  
185 distinguish neoplastic and residual normal cells, and (5) percentages of positive events may be redundant and may not  
186 contribute to clarity and easy interpretation of the results. Reporting percentages of positive events in different cell  
187 subpopulations, identified upon light scatter or immunophenotypic features (for instance high FSC low SSC cells, or  
188 CD21 positive cells) may further improve accuracy of the report.

189  
190 In contrast, a descriptive report may better focus on neoplastic cells, is clearer and easier to interpret, avoids  
191 redundancies and may provide information on co-expression, aberrant pattern(s) and quantitative expression.  
192 However, it may be less objective since it is often biased by the interpretation and experience of the specialist. It is  
193 recommended that objective (percentage of positive cells) and subjective (i.e., descriptive interpretation based on  
194 experience) statements be clearly identifiable in the report. The descriptive report should be conversational and may  
195 easily include data from other laboratory tests (cytological review, molecular clonality assessment, CBC, etc).

196  
197 Consensus was reached regarding the necessity to include both parts in the FC report, with an emphasis on the  
198 conversational, descriptive part. Data such as the percentages of positive cells should be reported in parentheses or in a  
199 table attached to the written report. Data about cells not considered important for the tumor subtype (for instance  
200 myeloid cells in lymphomas, T cell subsets in B cell neoplasms, residual lymphoid population in AML) should be  
201 reported, but with an effort to clearly identify them as additional information (in the conversational part) in order to  
202 avoid confusion and redundancies. These data could be discussed in more detail if they have been shown to be related  
203 to immunity against the tumor or have possible prognostic meaning based on published research.

### 204 205 **Minor issues**

#### 206 207 **Information regarding the quality of the sample**

208  
209 Information regarding the quality of the sample is crucial for interpreting the results of analysis and identifying any  
210 possible sources of bias. Unanimous consensus was reached about the relevance of including information regarding the  
211 quality of the sample and the evaluation of viability and preservation of cells in the report, including the type of

212 technique used. This was considered mandatory information by the majority of the participants. These data may be  
213 derived from the evaluation of scatterplots, the evaluation of viable cells by using specific stains (propidium iodide,  
214 trypan blue, etc) or using other qualitative methods. Objective methods (propidium iodide or other stains) are  
215 preferable and results may be reported as percentage of viable cells or with a descriptive method. The technique used  
216 to assess the quality of the sample should preferably be specified in the final report.

217

### 218 **Gating strategies**

219

220 Although gating strategy used may be considered unnecessary information for clinicians and owners, this piece of  
221 information could be of use for specialists in order to better interpret results and for second opinions. Consensus was  
222 reached in considering it as relevant information to be possibly included in an FC report but no consensus was reached  
223 about the level of recommendation.

224

### 225 **Dot plot images**

226

227 Attaching images of dot plots to final reports could be of some use to other specialists to better understand gating  
228 strategies. However, they may be of limited use and difficult to interpret for most users (clinicians and pet owners) and  
229 may lead to potential misinterpretation. In addition, the choice of plots (histogram vs. dot vs. tridimensional) is not  
230 standardized and the results of all the antibodies used are not easily summarized in a few images. The routine inclusion  
231 of dot plots in a final report was not favored. Images or preferably, fcs files should be kept and can be provided upon  
232 request.

233

### 234 **Reporting absolute numbers**

235

236 Reporting absolute numbers of leukocyte subpopulations may be done by directly counting cells with the flow  
237 cytometer or by calculating them from flow cytometric percentages and complete blood count (CBC) data. Reporting  
238 absolute numbers could be useful mainly in blood, while they are probably of limited importance in bone marrow and  
239 lymph node aspirates in which relative percentages (out of CD45 positive cells or total cells) are much more important.  
240 When absolute numbers are reported, laboratory specific reference intervals should be provided.

241

### 242 **Cutoff value**

243

244 The cutoff value for considering a neoplastic population positive or negative for a specific antibody is another important  
245 issue. The determination of the percentage of positive neoplastic cells depends on the appropriate negative controls  
246 used and may be variable among observers. The use of isotype or fluorescence minus one (FMO) controls is strongly  
247 encouraged to correctly define background staining and fluorescence spillover. An internal control (biological  
248 comparison control) i.e. a negative population of cells from residual or non-neoplastic cells, is also mandatory to  
249 correctly define a cutoff value. However, for the sake of clarity, the results of controls should not be included in the final  
250 report. Some authors [13] suggested 20–30% as the lower limit to define a population as positive to a specific antigen  
251 but this value has been reconsidered in human medicine. Other authors reported positivity as <10% of cells = low, 10–  
252 50% intermediate, >50% = high. No consensus was reached about a cutoff percentage value to define a cell population  
253 as positive, even though the majority of participants identified 20–30% as an acceptable value. The cutoff of positivity  
254 may depend upon the tissue analyzed (lymph node vs. peripheral blood vs. bone marrow) and the antigen investigated.  
255 The issue of controls and cut-off values was considered a crucial issue but beyond the scope of the present consensus  
256 paper (reporting flow cytometry results). Owing to its critical importance, this issue will be the focus of a future  
257 consensus document of the ECLN. Regarding this article, consensus was reached about the need to include appropriate  
258 controls in the flow cytometric procedure, but inclusion of the control results in the final report is not encouraged.

259 Reporting quantitative antigen expression

260

261 This may be useful for common lineage antigens (CD45, CD44, CD18) or activation antigens (Ki67, MHC II) and may  
262 provide information about maturation status, aberrant expression, or prognosis. In dogs, common lineage antigens  
263 have been reported to show different expression intensities in hematopoietic subsets and different maturation and

264 activation stages [14, 15]. MHC II has been reported to be associated with prognosis in canine B cell lymphoma [16].  
265 Ki67 has been reported to be useful in differentiating low and high grade lymphomas [17]. Quantitative aberrancies  
266 have been reported in different lymphoma subtypes [18]. Reporting intensities of antigen expression may be useful for  
267 markers with possible prognostic significance or for those expressed differently than expected.

268  
269 Quantitative expression is generally reported categorically as bright or dim but this may be subjective and poorly  
270 repeatable. Some authors reported antigen expression as bright or dim when a difference of at least 15% in  
271 fluorescence channels was present in the neoplastic population compared to the residual population of the same  
272 lineage [18]. This method is repeatable among different laboratories but it is quite complicated and it requires a clear  
273 identification of non-neoplastic residual cells of the same phenotype. Other authors [16] compared Mean Fluorescence  
274 Index (MFI) of a specific antigen (MHC II) in neoplastic cells to that derived from a cohort of neoplastic cases; expression  
275 was reported as “dim” if MFI was lower than the 15th percentile of all MFI calculated from a large series of canine  
276 lymphomas. This method is probably easier but it requires a different specific standardization from each laboratory to  
277 define the adequate cutoff value for fluorescence and a large caseload to calculate appropriate reference values. In  
278 addition, the expression of some antigens could be compared with those of a non-neoplastic reference population  
279 clearly identifiable in the sample (such as neutrophils in peripheral blood) [14]. This method is easy to perform and does  
280 not require any specific standardization but it is quite subjective and it is based on the assumption that antigen  
281 expression remains constant in the reference population. Finally, antigen expression could be accurately quantitated  
282 using a curve with calibrated beads but this method is expensive and difficult to apply to clinical/diagnostic conditions.

283  
284 In human medicine, the issue of antigen expression is also crucial. Intensity of staining for some antigens may be  
285 expressed as bright, dim, or negative, and according to their distribution as heterogeneous or homogeneous. Some  
286 authors also suggest possible reporting of expression as weak or strong. Strong refers to unequivocal positivity (not  
287 necessarily to bright expression) [8]. Other authors suggest defining intensity of some antigens as low (when the  
288 histogram is significantly different but not easily separable from the negative control), middle (when the fluorescence  
289 peak is contiguous to the negative control but completely distinguishable from it), or high (when the fluorescence peak  
290 is two or three logarithmic decades higher than the negative control) [19]. Some antigens (such as ZAP-70 protein in  
291 human CLL) are reported comparing MFI of neoplastic cells to those of T cells in the same sample. This method is  
292 reported as easy to perform and optimal for accurately predicting outcome in CLL [20].

293  
294 Because no definitive rules on the best way to report fluorescence intensity of antigens in FC reports of canine  
295 hematopoietic neoplasms have been generated to date, some recommendations are proposed: (1) antigen expression  
296 may be preferentially expressed as dim or bright and data on distribution of the antigen (homogeneous vs.  
297 heterogeneous) should be provided only if useful to discriminate between normal and neoplastic populations, to define  
298 subtype, to track infiltration/residual disease of neoplastic cells (when quantitative aberrant patterns are present) or to  
299 define prognosis; (2) when this quantitation is reported, the definition of neoplastic cells as dim or bright should be well  
300 standardized for each antigen and consistent with methods from published references; (3) in the absence of specific  
301 published references, the criteria used for defining quantitative antigen expression (dim vs. bright) could be provided in  
302 supplementary notes, together with the putative biological meaning; (4) when possible, quantitative expression of  
303 antigens should be compared with the closest normal hemic population; (5) quantitative findings that are irrelevant to  
304 clinical importance (i.e., not involving staging, prognosis, minimal residual disease, etc.) should be avoided; (6) data  
305 regarding in-progress studies should be omitted from the diagnostic report until they complete the peer-review and  
306 publication process. If necessary, references of published FC entities could be provided in the notes.

### 307 308 **Reporting aberrant patterns**

309  
310 Qualitatively and or quantitatively aberrant patterns have been reported in several studies in different lymphoma  
311 subtypes in dogs [18, 21, 22] and may differ from the human counterparts. Their importance is far from completely  
312 elucidated but, in some cases, specific aberrant patterns may be useful to define specific subtypes, or to accurately  
313 track neoplastic infiltration in tissues and to detect minimal residual disease. In particular, a specific subtype of canine  
314 lymphoma with peculiar aberrant patterns different from its human counterpart, T zone lymphoma, is well recognized.  
315 This indolent T cell lymphoma subtype is not uncommon in the dog, in contrast to people, and exhibits a characteristic



316 decreased expression of CD45 and frequently the concurrent aberrant expression of CD21 [1, 2]. The likelihood of  
317 detecting an aberrant phenotype is linked to the number of antibodies used and to the type of labeling and analysis  
318 performed (mono- vs. multi-color). Although evidence of the prognostic role of aberrancies in canine hematopoietic  
319 neoplasms is largely still lacking, it is likely that some specific aberrancies could have a correlation with biological  
320 behavior, similar to what has been reported in human medicine [23-28]. Clinicians are often interested in the presence  
321 of aberrant patterns, although they may tend to overestimate their meaning. The report of percentages of positive cells  
322 alone may miss the detection of specific aberrant patterns. The presence of a specific aberrant pattern in neoplastic  
323 cells should be indicated in the descriptive part of the report if it may be useful to document the infiltration of  
324 neoplastic cells in organs, to monitor therapy and minimal residual disease, to facilitate the early detection of relapse or  
325 if it may have a specific biological meaning (prognosis, response to therapy, etc).

326  
327 Adding a statement to the descriptive part of the report regarding possible FC marker(s) to check infiltration or monitor  
328 follow-up and detect relapse may be helpful and should be encouraged. These markers may include single labeling (for  
329 instance CD34+ cells in acute leukemias), multiple labeling (e.g., CD3+ CD45- cells for T zone lymphomas) or  
330 fluorescence and morphological aspects together (e.g., large CD21+ cells for DLBCL).

### 331 332 **Integration of other clinical data**

333  
334 Moving toward an integrated report including hematologic, cytologic, histopathologic, immunohistochemical, and  
335 molecular biologic data is a major goal. However, the availability of results will influence the possibility of a report  
336 integrating all of the laboratory results. An integrated report including hematologic, cytologic, and FC results while  
337 waiting for results of other ancillary techniques may be a good compromise and is encouraged.

### 338 339 **Proposal of a Possible Template for Reporting FC Results**

340  
341 According to the previously discussed issues, the proposed report for FC immunophenotyping of canine hematopoietic  
342 neoplasms should include several sections.

- 343
- 344 1. Laboratory identification
- 345 2. Patient identification
- 346 3. Type and quality of the sample(s)
- 347 4. Sample preparation and staining
- 348 5. Percentages of positive cells
- 349 6. Descriptive report
- 350 7. Diagnosis and interpretation
- 351 8. Comments and references
- 352 9. Signatures
- 353

354 For each section some mandatory, recommended and additional information was identified (Table 1).

### 355 356 **Conclusion**

357  
358 The present article is a first step toward standardization of the flow cytometric approach for canine hematopoietic  
359 neoplasms among different institutions and countries. It should help to provide a more accurate report to users and  
360 support the use of flow cytometric immunophenotyping in the diagnostic algorithm for canine lymphoma and leukemia.  
361 The creation of consensus documents on other important issues, including preprocessing, instrument standardization  
362 and maintenance, controls and cut-offs, and suggested antibody panels is an ongoing process for the flow cytometry  
363 panel of the ECLN.

### 364 365 **Acknowledgements**

366  
367 The authors thank the members of the ECLN flow cytometry panel: Drs Ugo Bonfanti, Juan Borrego, Francesco Cian,

368 Jane Dobson, Mariaelena Gelain, Maciej Guzera, Josep Pastor, Roger Powell, Andrzej Rapak, Elisabeth Villiers, and the  
369 members of the ECLN therapy working group who responded to the survey. The European Canine Lymphoma Network  
370 is supported by Solaris Healthcare, Chiasso, CH.  
371

372  
373  
374  
375  
376  
377  
378  
379  
380  
381  
382  
383  
384  
385  
386  
387  
388  
389  
390  
391  
392  
393  
394  
395  
396  
397  
398  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421  
422  
423

## References

1. Seelig DM, Avery P, Webb T, Yoshimoto J, Bromberek J, Ehrhart EJ, Avery AC. Canine T-zone lymphoma: Unique immunophenotypic features, outcome, and population characteristics. *J Vet Intern Med* 2014;v28:878–886.
2. Martini V, Poggi A, Riondato F, Gelain ME, Aresu L, Comazzi S. Flow-cytometric detection of phenotypic aberrancies in canine small clear cell lymphoma. *Vet Comp Oncol* 2015;13:281–287.
3. Vernau W, Moore PF. An immunophenotypic study of canine leukemias and preliminary assessment of clonality by polymerase chain reaction. *Vet Immunol Immunopathol* 1999;69:145–164.
4. Comazzi S, Gelain ME, Martini V, Riondato F, Miniscalco B, Marconato L, Stefanello D, Mortarino M. Immunophenotype predicts survival time in dogs with chronic lymphocytic leukemia. *J Vet Intern Med* 2011;25:100–106.
5. Marconato L, Martini V, Aresu L, Sampaolo M, Valentini F, Rinaldi V, Comazzi S. Assessment of bone marrow infiltration diagnosed by flow cytometry in canine large B cell lymphoma: Prognostic significance and proposal of a cut-off value. *Vet J* 2013;197:776–781.
6. Aresu L, Aricò A, Ferraresso S, Martini V, Comazzi S, Riondato F, Giantin M, Dacasto M, Guadagnin E, Frayssinet P, et al. Minimal residual disease detection by flow cytometry and PARR in lymph node, peripheral blood and bone marrow, following treatment of dogs with diffuse large B-cell lymphoma. *Vet J* 2014;200:318–324.
7. Gustafson MP, Lin Y, Ryder M, Dietz AB. Strategies for improving the reporting of human immunophenotypes by flow cytometry. *J Immunother Cancer* 2014;2:18.
8. Hrušák O, Basso G, Ratei R, Gaipa G, Luria D, Mejstříková E, Karawajew L, Buldini B, Rozenthal E, Bourquin JP, et al. Flow diagnostics essential code: A simple and brief format for the summary of leukemia phenotyping. *Cytometry B Clin Cytom* 2014;86:288–291.
9. Braylan RC, Atwater SK, Diamond L, Hassett JM, Johnson M, Kidd PG, Leith C, Nguyen D. U.S.-Canadian Consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: Data reporting. *Cytometry* 1997;30:245–248.
10. Wood BL, Arroz M, Barnett D, DiGiuseppe J, Greig B, Kussick SJ, Oldaker T, Shenkin M, Stone E, Wallace P. 2006 Bethesda International Consensus recommendations on the immunophenotypic analysis of hematology neoplasia by flow cytometry: Optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. *Cytometry B Clin Cytom* 2007;72(Suppl 1):S14–S22.
11. Comazzi S, Marconato L, Argyle DJ, Aresu L, Stirn M, Grant IA, Guscetti F, Hendrickx T, Ibisch C, Lawrence JA, et al. European Canine Lymphoma Network The European canine lymphoma network: A joining initiative to generate consensus guidelines for the diagnosis and therapy in canine lymphoma and research partnership. *Vet Comp Oncol* 2015;13:494–497.
12. Loblaw DA, Prestrud AA, Somerfield MR, Oliver TK, Brouwers MC, Nam RK, Lyman GH, Basch E, American Society of Clinical Oncology Clinical Practice Guidelines. American Society of Clinical Oncology Clinical Practice Guidelines: Formal systematic review-based consensus methodology. *J Clin Oncol* 2012;30:3136–3140.
13. Bain BJ, Barnett D, Linch D, Matutes E, Reilly JT, General Haematology Task Force of the British Committee for Standards in Haematology (BCSH), British Society of Haematology. Revised guideline on immunophenotyping in acute leukaemias and chronic lymphoproliferative disorders. *Clin Lab Haematol* 2002;24:1–13.
14. Comazzi S, Gelain ME, Riondato F, Paltrinieri S. Flow cytometric expression of common antigens CD18/CD45 in blood from dogs with lymphoid malignancies: A semi-quantitative study. *Vet Immunol Immunopathol* 2006;112:243–252.
15. Gelain ME, Martini V, Giantin M, Aricò A, Poggi A, Aresu L, Riondato F, Dacasto M, Comazzi S. CD44 in canine leukemia: Analysis of mRNA and protein expression in peripheral blood. *Vet Immunol Immunopathol* 2014;159:91–96.
16. Rao S, Lana S, Eickhoff J, Marcus E, Avery PR, Morley PS, Avery AC. Class II major histocompatibility complex expression and cell size independently predict survival in canine B-cell lymphoma. *J Vet Intern Med* 2011;25:1097–1105.
17. Poggi A, Miniscalco B, Morello E, Comazzi S, Gelain ME, Aresu L, Riondato F. Flow cytometric evaluation of ki67 for the determination of malignancy grade in canine lymphoma. *Vet Comp Oncol* 2015;13:475–480.
18. Gelain ME, Mazzilli M, Riondato F, Marconato L, Comazzi S. Aberrant phenotypes and quantitative antigen expression in different subtypes of canine lymphoma by flow cytometry. *Vet Immunol Immunopathol*

- 424 2008;121:179–188.
- 425 19. Del Vecchio L, Brando B, Lanza F, Ortolani C, Pizzolo G, Semenzato G, Basso G, Italian Society for Cytometry.  
426 Recommended reporting format for flow cytometry diagnosis of acute leukemia. *Haematologica* 2004;89:594–598.
- 427 20. Smolej L, Vroblava V, Motyckova M, Jankovicova K, Schmitzova D, Krejsek J, Maly J. Quantification of ZAP-70  
428 expression in chronic lymphocytic leukemia: T/B-cell ratio of mean fluorescence intensity provides stronger  
429 prognostic value than percentage of positive cells. *Neoplasma* 2011;58:140–145.
- 430 21. Wilkerson MJ, Dolce K, Koopman T, Shuman W, Chun R, Garrett L, Barber L, Avery A. Lineage differentiation of  
431 canine lymphoma/leukemias and aberrant expression of CD molecules. *Vet Immunol Immunopathol* 2005;106:179–  
432 196.
- 433 22. Guija de Arespacochaga A, Schwendenwein I, Weissenböck H. Retrospective study of 82 cases of canine lymphoma  
434 in Austria based on the Working Formulation and immunophenotyping. *J Comp Pathol* 2007;136:186–192.
- 435 23. Schmidt CJ, Domenico L, Ward P, Barcos MP, Stewart CC. Aberrant antigen expression detected by multiparameter  
436 three color flow cytometry in intermediate and high grade B-cell lymphomas. *Leuk Lymph* 1999;34:539–544.
- 437 24. Dunphy CH, Gardner LJ, Manes JL, Bee CS, Taysi K. CD30+ anaplastic large-cell lymphoma with aberrant expression  
438 of CD13: Case report and review of the literature. *J Clin Lab Anal* 2000;14:299–304.
- 439 25. Sánchez ML, Almeida J, Vidriales B, López-Berges MC, García-Marcos MA, Moro MJ, Corrales A, Calmuntia MJ, San  
440 Miguel JF, Orfao A. Incidence of phenotypic aberrations in a series of 467 patients with B chronic  
441 lymphoproliferative disorders: Basis for the design of specific four-color stainings to be used for minimal residual  
442 disease investigation. *Leukemia* 2002;16:1460–1469.
- 443 26. Kampalath B, Barcos MP, Stewart C. Phenotypic heterogeneity of B cells in patients with chronic lymphocytic  
444 leukemia/small lymphocytic lymphoma. *Am J Clin Pathol* 2003;119:824–832.
- 445 27. Went P, Agostinelli C, Gallamini A, Piccaluga PP, Ascani S, Sabattini E, Bacci F, Falini B, Motta T, Paulli M, et al.  
446 Marker expression in peripheral T-cell lymphoma: A proposed clinical-pathologic prognostic score. *J Clin Oncol*  
447 2006;24:2472–2479.
- 448 28. Mayson E, Saverimuttu J, Cartwright K. CD5-positive follicular lymphoma: Prognostic significance of this aberrant  
449 marker? *Intern Med J* 2014;44:417–422.
- 450

451 Table 1. Results of the consensus survey among ECLN flow cytometry panel on recommended information in different  
 452 sections of a canine hematopoietic neoplasm flow cytometry report. Each piece of information was considered  
 453 “relevant” if more than 75% of responders classified it as “mandatory”, “recommended” or “additional”.  
 454 Recommendations for each piece of information reflect the category at which > 50% of responders concurred. Failure  
 455 to achieve these thresholds resulted in a designation of no consensus. Relevancy or recommendation scores of 100%  
 456 are in bold.

		Percentage of agreement (%)				Relevance of information	Recommendation
		mandatory	recommended	additional	irrelevant		
Laboratory identification	Name of the laboratory	100	0	0	0	Relevant	Mandatory
	Postal address	53	27	13	7	Relevant	Mandatory
	Telephone number	60	40	0	0	Relevant	Mandatory
	e-mail contact	60	40	0	0	Relevant	Mandatory
	Web page	13	47	27	13	Relevant	No consensus
	Authorization number or licenses	20	40	27	13	Relevant	No consensus
Patient identification	Date of the report	93	7	0	0	Relevant	Mandatory
	Date of analysis	64	36	0	0	Relevant	Mandatory
	Internal ID code	60	40	0	0	Relevant	Mandatory
	Owner's name	80	13	7	0	Relevant	Mandatory
	Referring physician name and institution	53	40	7	0	Relevant	Mandatory
	Species	93	7	0	0	Relevant	Mandatory
	Breed	67	33	0	0	Relevant	Mandatory
	Gender	60	40	0	0	Relevant	Mandatory
	Age	73	27	0	0	Relevant	Mandatory
	Patient name	60	27	7	7	Relevant	Mandatory
	Previous therapy	33	13	54	0	Relevant	Additional
	Clinical history	40	20	40	0	Relevant	No consensus
	Other laboratory results (CBC, Diff)	27	40	33	0	Relevant	No consensus
Type and quality of the sample	Type of tissue(s)	93	7	0	0	Relevant	Mandatory
	Type of sample (aspirate, biopsy, blood, fluid, etc)	73	27	0	0	Relevant	Mandatory
	Quality of the sample (estimated)	73	27	0	0	Relevant	Mandatory
	Sampling data	73	27	0	0	Relevant	Mandatory
	Percentage of viable cells	26	60	13	0	Relevant	Recommended
	Technique used for viability estimation	13	73	13	0	Relevant	Recommended
	Specimen number	33	53	13	0	Relevant	Recommended
Cell preparation and staining	Antibodies used	87	13	0	0	Relevant	Mandatory

	Cell preparation (whole sample, scraping, Ficoll, RBC lysis)	20	53	27	0	Relevant	Recommended
	Antibody clone	0	13	60	27	No consensus	Additional
	Fluorochrome combination	0	13	60	27	No consensus	Additional
	Composition of tubes	0	7	73	20	Relevant	Additional
	Type of controls	0	27	60	13	Relevant	Additional
	Instrumentation	0	13	53	33	No consensus	Additional
	Expected positivities for each antibody	13	33	40	13	Relevant	No consensus
Descriptive report	Qualitative description of immunophenotype of cells of interest	78	14	7	0	Relevant	Mandatory
	Comment on quantitative expression of relevant markers	53	27	20	0	Relevant	Mandatory
	Aberrant patterns	67	33	0	0	Relevant	Mandatory
	Information about staging	7	80	13	0	Relevant	Recommended
	Information about residual cells	0	73	27	0	Relevant	Recommended
	Qualitative description of scatter aspects of cells of interest	47	40	13	0	Relevant	No consensus
Percentage of positive cells	Percentage of positive cells in gated population	53	47	0	0	Relevant	Mandatory
	Percentage of positive cells in whole population	29	57	7	7	Relevant	Recommended
	Intensity of staining (dim vs bright, homogeneous)	20	60	20	0	Relevant	Recommended
	Absolute count on positive cells (in peripheral blood only)	20	53	20	13	Relevant	Recommended
	CD4/CD8 ratio	7	27	53	13	Relevant	Additional
	Representation of histogram/plot	7	20	60	13	Relevant	Additional
	Fluorescence index (quantitative)	0	40	47	13	Relevant	No consensus
	Gating procedure	13	33	33	20	Relevant	No consensus
	Reference intervals for each antigen	7	40	47	7	Relevant	No consensus
Diagnosis and interpretation	Diagnosis of immunophenotype	100	0	0	0	Relevant	Mandatory
	Lymphoma subtype (tentative)	53	40	7	0	Relevant	Mandatory
	Stage	20	60	7	13	Relevant	Recommended

	Suggested FC markers for monitoring stage and MRD	0	67	33	0	Relevant	Recommended
	Possible prognostic factors	27	53	20	0	Relevant	Recommended
	Grade (high vs low) (tentative)	40	40	20	0	Relevant	No consensus
<b>Comments</b>	Clinical and outcome information	0	64	36	0	Relevant	Recommended
	Additional tests suggested	0	53	40	7	Relevant	Recommended
	References	0	47	53	0	Relevant	Additional
<b>Signatures</b>	Party responsible for the service	73	20	7	0	Relevant	Mandatory
	Flow cytometrist	33	47	20	0	Relevant	No consensus