Cytocentrifuge preparation in veterinary cytology: a quick, simple, and affordable manual method to concentrate low cellularity fluids

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Key Words

Cat, cytocentrifuge, cytology, cytospin, dog

Background: Smears prepared by cytocentrifugation, the so-called cytospins, are widely used in human and veterinary cytology. However, the high cost has hampered the availability of commercial cytospin centrifuges in some veterinary clinics and laboratories. Nevertheless, cytospins are important for evaluating fluids with very low cellularity such as cerebrospinal fluid (CSF) or bronchoalveolar lavage fluid (BALF).

Objectives: The aim of this study was to devise and test the use of a low-cost, in-house manual cytocentrifuge to obtain cytospin preparations.

Methods: Twenty-two fluid samples (including CSF and BALF) were collected from dogs and cats. These were processed in a conventional cytocentrifuge and in an in-house, manual centrifuge (salad spinner). The cytospins obtained by the 2 methods were compared by scoring cellularity, number of cells per field, hemodilution, cell preservation, and proportion of ruptured cells. Additionally, cell number and size were compared by morphometry. Differences between the automated and manual method were statistically assessed.

Results: The morphology and cellular detail of cytospin preparations produced by both methods were identical. There was an almost perfect agreement for cellularity, number of cells per HPF, hemodilution and cell preservation (kappa ≥ 0.85), and a moderate agreement for the amount of ruptured cells. Cell recovery was comparable (including in CSF and BALF).

Conclusions: The manual cytocentrifuge produced cytospins with similar cell yield as the automated cytocentrifuge. Considering the low cost and portability, this new method should be particularly useful for cytologic diagnosis in small clinics, developing countries, and in field studies

## Introduction

The study of fluid samples (ie, effusions, cerebrospinal fluid [CSF], bronchoalveolar lavage fluid [BALF], or urine) is relevant for the diagnosis, treatment, and follow-up of inflammatory and neoplastic conditions both in human and veterinary medicine. Except for CSF, most veterinary practitioners collect such samples on a weekly basis.[1] In low cellular fluids, it is often mandatory to concentrate the cells for an adequate evaluation of cellular morphology. Smears prepared in cytocentrifuges, the so-called cytospin smears or cytospins, are widely used in human and veterinary cytology to concentrate cells.[2] In cytospins, cells

are automatically spun directly onto a small area of a slide by slow centrifugation, enabling a fast and reproducible microscopic evaluation. Nowadays, cytospin preparations are considered the best method for cell concentration in veterinary medicine [3,4], but cytospins are less used in veterinary practice, which is probably due to the relatively high price of automated cytocentrifuges.5 Although such low cellularity fluid samples are often sent by mail to laboratories, the rapid cell degeneration may compromise a cytologic diagnosis in subsequent cytocentrifuge preparations. Specifically, cells may degenerate in as few as 24 hours resulting in erroneous differential counts in inflammatory samples[6] and compromised assessment of neoplastic criteria.[7] This is especially true for CSF and BALF, which should be processed, ideally, within one or 2 hours after sampling, respectively.[8,9] Therefore, an affordable, in-house, and quick method for obtaining cytospins from fluid samples would be of value to veterinary practitioners. The aim of this study was to devise a new and lowcost technique to generate cytospin preparations providing adequate cellularity and morphology, comparable with samples processed in an automated cytocentrifuge.

## Materials and Methods

### Cytologic specimens

Twenty-two fluid samples from dogs (20 cases) and cats (2 cases) received for diagnostic evaluation were studied. These comprised 11 cavitary effusions (6 pleural, 3 peritoneal, and 2 pericardial), 8 CSFs, 2 BALFs, and one urine. An equal aliquot of the specimen was processed in a commercially available automated cytocentrifuge (Statspin Cytofuge 2 Inc., Norwood, MA, USA) and by an alternative manual method described below. Processing occurred within 30 min (CSF) or maximum one h after sample collection.

### Cytospins produced by automated cytocentrifugation

In both procedures, 200 lL of the specimen were pipetted into a reusable cell concentrator (VWR cat: 720-1972; CytoSep, Beloeil, QC, Canada) attached to a glass slide layered with a disposable filter with a central hole of 7.25 mm diameter (VWR cat 720-1973), and held together with clips (StatSpin cat: FFCL). It was then centrifuged for 6 min in a Statspin Cytofuge 2 at 140g (1600 rpm) in all cases except for CSF (8 min at 40g [850 rpm]), following the manufacturer's recommendations. The obtained cytospins served as reference for the alternative manual procedure.

### Cytospins produced by the alternative manual method

The second specimen was placed in a modified commercial salad spinner (26 cm diameter, Zyliss cat: 15201; Diethelm Keller brands, Zurich, Switzerland) (Figure 1). This kitchen device is accelerated by pulling a handle continuously to half distance 90-100 times per min (controlled by a wall watch). On average, the basket rotated at 127g (1150 rpm) for 5 min as measured by a digital tachometer (DT-2234C; Rinch Industrial, Shanghai, China, accuracy 1 rpm). The same duration and rotation were followed for all fluid samples (including CSF). Styrofoam cushions of 5, 9, 3, 9, 2 cm were fitted to the basket with rubber bands (Figure 2), which also held the cell concentrators in place. All slides were stained with a commercial Romanowsky-type stain (Hemacolor; Merck, Darmstad, Germany) and mounted with mounting media (Cover-quick 2000; VWR Chemicals, Fontenay-sous-bois, France).

### Qualitative and quantitative comparison between samples

For the comparison of the 2 methods, slides were coded and scored blindly by an experienced board-certified cytopathologist (MC). Samples were scored on a 1-3 scale for cellularity (1 = low, 2 = moderate, 3 = high); cell preservation (1 = poor, 2 = moderate, 3 = good); and proportion of ruptured cells per 960 objective HPF (1 = ≤ 10%, 2 = > 10 < 50%, 3 = ≥ 50%). A semiquantitative evaluation of hemodilution (1 = ≤10 RBC, 2 = > 10 and < 100 RBC, 3 = ≥ 100 RBC per HPF) and of the number of nucleated cells per HPF (1 = ≤ 10, 2 = > 10 and < 100, 3 = ≥ 100 per HPF) was also assessed.For the comparison of cell number and size, we used morphometry. Photos from a field in each quadrant and the central part of the circular area

were taken with a 9100 objective with oil and the average cell diameter assessed with the ImageJ software 1.47v (http://imagej.nih.gov/ij). As neoplastic cells usually display anisocytosis, only the size of erythrocytes and neutrophils was considered (25 cells per case, on average). Additionally, the number of all nucleated cells per field was assessed. For the statistical analysis, the software SPSS18 (IBM, Armonk, NY, USA) was used. Differences between scores were assessed using the Wilcoxon signed-rank test, with a Bonferroni correction, and the statistical significance was set at $P < .05$. The agreement between the 2 cytospin methods was assessed with kappa statistics. For interpreting the strength of agreement, the following standards were considered: ≤0.40 = poor, 0.41-0.60 = moderate, 0.61-0.80 = good, and 0.81-1 = almost perfect.[10] For the differences in the cell diameters and number of cells per field, the Mann-Whitney U test was applied.

## Results

The investigated specimens covered all potential pathologies, including inflammatory and neoplastic cytologic diagnoses as well as normal and transudative fluids. The one urine sample only contained struvite crystals. Processing samples in both spinning devices resulted in good quality cytospins, with cells distributed over the circular area roughly corresponding to the area covered by the 94 objective (Figure 3). The manual method was as straightforward to use as the automated cytocentrifuge: inserting and removing the cell concentrators was easy, and pulling the handle during 5 min was manageable. In general, cytospins obtained by the 2 methods were comparable (Table 1, Figure 4). The size of erythrocytes and neutrophils was similar in both methods (6.2   1.0 lm and 5.6  0.7 lm with manual and automated cytospins, respectively forerythrocytes, and 11.2  2.1 lm and 12.2  2.1 lm with manual and automated cytospins, respectively for ). The median of nucleated cells per HPF was 11 (range 0-22) and 13 (range 0-46) in manual and automated cytospins, respectively, without statistically significant differences. The semi-quantitative analysis also showed no statistically significant differences between samples prepared by both methods (Table 1). An almost perfect agreement was observed for cellularity with a kappa of 0.85 (95% CI: 0.69-1.00). Indeed, a kappa of 0.92 (CI: 0.85-1.00) was estimated for the number of cells per HPF. The hemodilution and cell preservation were similar with both methods (kappa = 0.93 [CI: 0.81-1.00] and 0.92 [CI: 0.84-1.00], respectively). A moderate agreement existed for the number of ruptured cells (kappa = 0.47 [CI: 0.29 to 1.00]).

## Discussion

Cytocentrifugation, either by a commercially available dedicated automated centrifuge or a manual converted salad spinner, was compared using qualitative and quantitative morphologic microsopic criteria. Speed, simplicity, and low cost are relevant criteria for the widespread use of any diagnostic method. In cytocentrifugation, the relatively high cost of the equipment has hampered cytoconcentration in some veterinary environments[5], and automated cyocentrifugation has been confined to large diagnostic laboratories. We developed a simple and reliable manual cytocentrifugation method that generated cytospins in the same amount of time and providing comparable cytologic quality as the automated cytocentrifuge, at a fraction of the cost (Zyliss salad spinner about 34$, Statspin Cytofuge 2 at least 3600$). As cell concentrators and metallic clips are reusable, filters are fairly inexpensive and similar devices substituting for official brand ware provide even less expensive options fulfilling the purpose (Figure 5), the overall manual approach is reasonably cheap. Fluids, especially of low cellularity, should be processed immediately after collection to avoid degenerative cellular artifacts. In the absence of a cytocentrifuge, sediments can be prepared with a conventional centrifuge after pouring off the supernatant and resuspending the cell pellet. However, this often leads to suboptimal results[3,8,11] as cells may be distorted and smudged while deposited and smeared onto a slide.[2] Moreover, smearing the very small cell button is impracticable in low cellular fluids, such as CSF or even BALF, in most instances. Gravity sedimentation chambers can be built with in-house material[3,12,13], but these require up to 60 minutes for cell sedimentation, and the resulting slides are usually of lower quality compared to cytocentrifuge preparations.[14] With the manual

cytocentrifugation method described in this report, veterinary practitioners can easily perform stable cytocentrifuge preparations suitable for shipping and evaluation by a reference laboratory, avoiding cellular degeneration and artifacts due to the long storage of low-protein fluids.[9] Manual hand-powered centrifuges have been developed for molecular biology[15] and hematology use[16], and this is the first report proposing an application in diagnostic cytology. Obviously, a manual centrifuge is not practical in large laboratories processing a high number of samples on a daily basis, but they are valuable in a small clinic or practice laboratory that occasionally needs to process such liquid samples. Moreover, the manual method described here has potential for field studies and is also applicable for samples in large animal medicine, such as BALF in horses[11] or even milk evaluation in dairy cows.[17]

In conclusion, the manual cytocentrifuge method seems a valuable option for processing low cellularity samples such as CSF and BALF. Considering the simplicity, adequate speed, and low cost, this method could be included in the toolbox of veterinarians devoted to cytologic diagnosis in very small or rural clinics, including developing countries and other field conditions.

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Figure 1. Manual cytocentrifugation method using a modified commercially available salad spinner (A), with a cord attached to a handle (B).

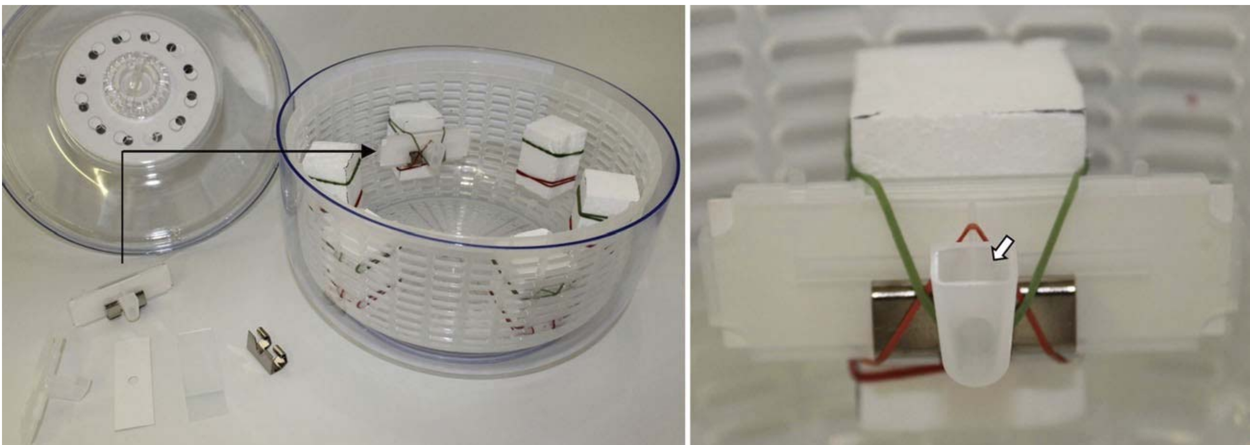

Figure 2. Manual cytocentrifugation using an adapted salad spinner. Up to 6 assembled cell concentrators with a slide and filter, each were accommodated inside a salad spinner using styrofoam cushions and rubber bands (A). The fluid was pipetted into the funnel of the concentrator (block arrow in B).

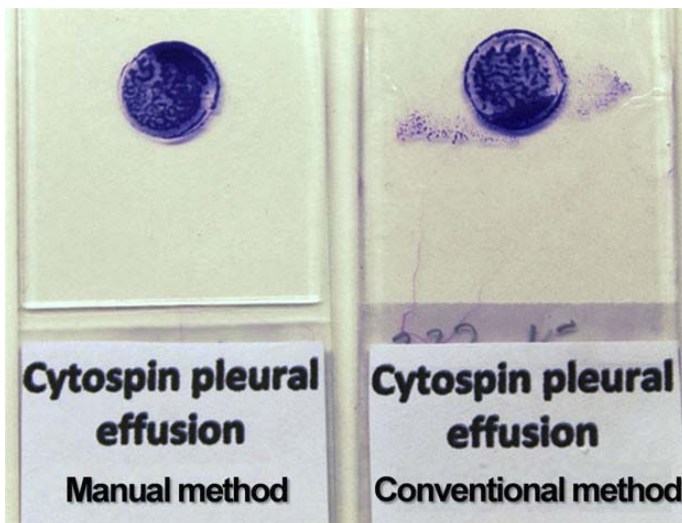


Figure 3. Macroscopic presentation of cytospins produced by the manual and automated cytocentrifuge methods.

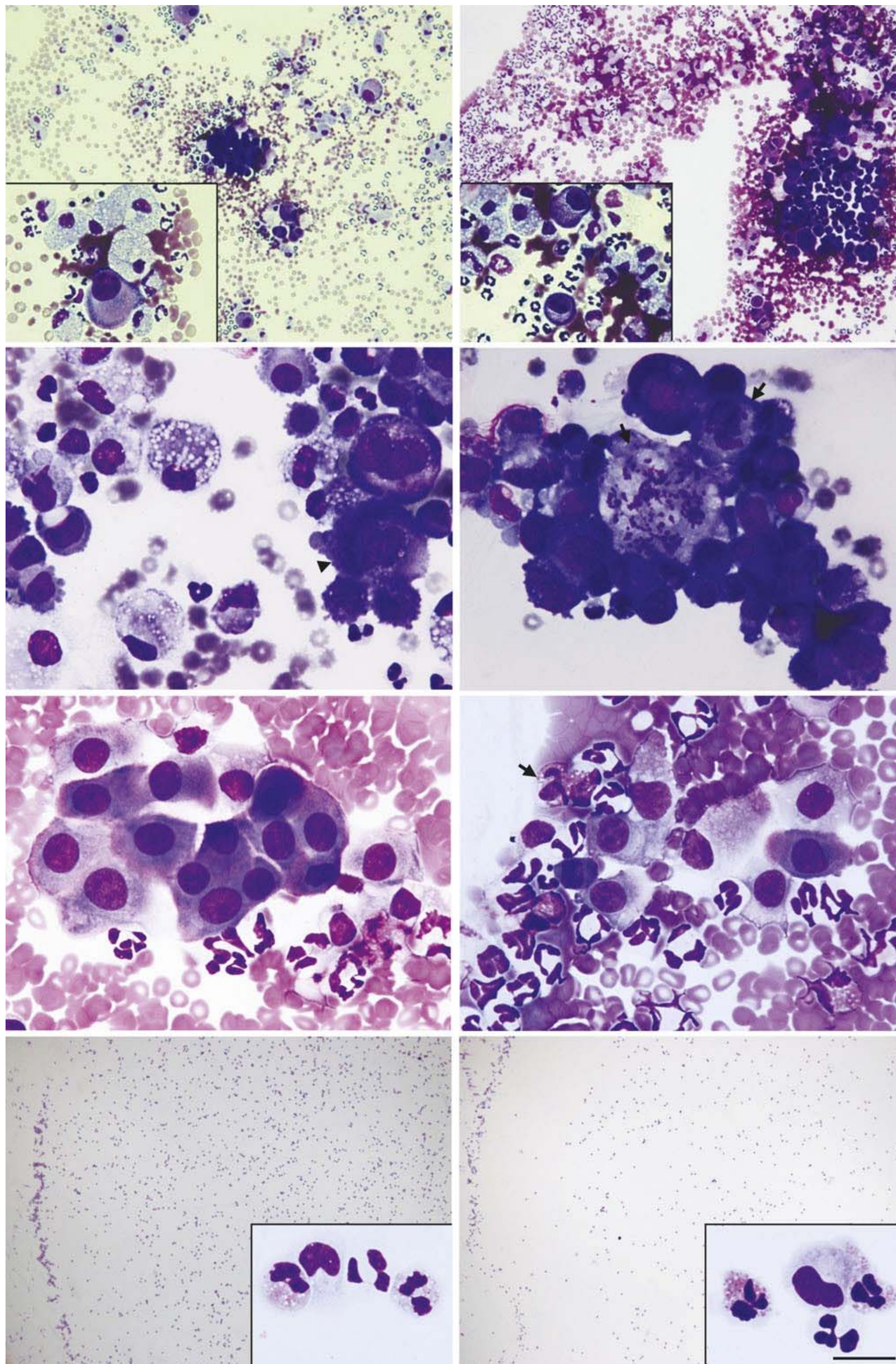

Figure 4. Cytospins generated by automated (A, C, E, G) and manual cytocentrifugation (B, D, F, H), Hemacolor. (A and B) Inflammatory pleural effusion from a dog. Neutrophils, macrophages, and mesothelial cells in a hemodiluted background [bar = 120 lm (inset 65 lm)]. (C and D) Neoplastic pleural effusion from a dog with a history of mammary gland adenocarcinoma. Anisocytosis, anisokaryosis, nuclear molding (arrowhead), and atypical mitotic figures (arrow) can be seen in automated (C) and manual (D) cytospins (bar = 25 lm). (E and F) Bronchoalveolar lavage fluid of a dog. Inflammatory cells, including eosinophils (arrows), appear along with ciliated and goblet cells in automated (E) and manual (F) cytospins (bar = 25 lm). (G and H) Cerebrospinal fluid with a moderate eosinophilic pleocytosis. Monocytoid cells, eosinophils, and neutrophils (inset) are similar in automated (G) and manual (H) cytospins (bar = 375 lm [inset = 30 lm]). Hemacolor.

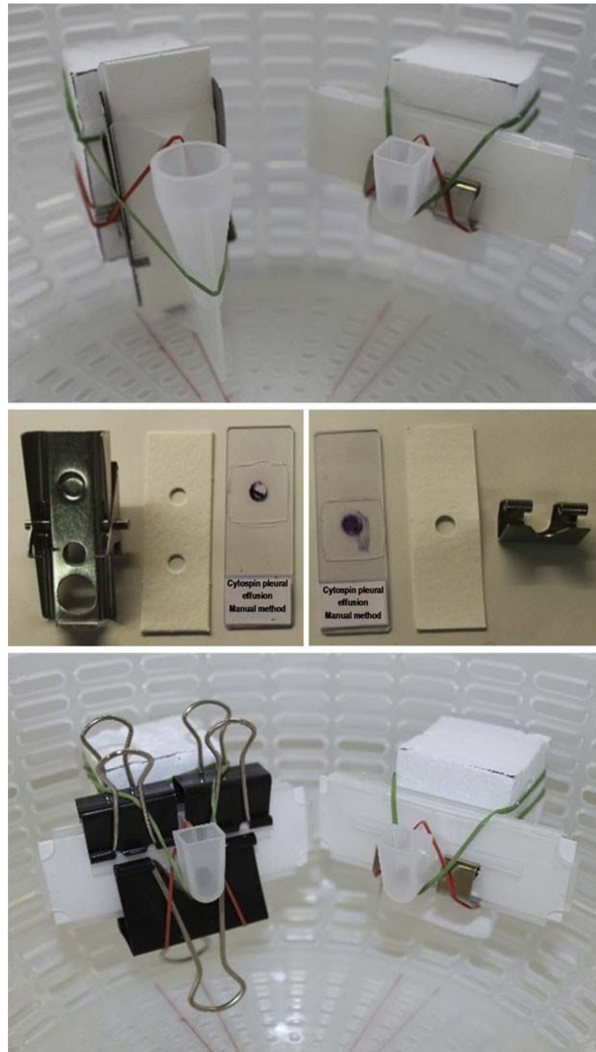


Figure 5. Cell concentrators, filters, and clips used for automated and manual cytocentrifugation methods by the main providers ([A left and B] Cytospin Shandon [A right, C] StatSpin Cytofuge 2). The metallic clips can also be replaced by adapted paper binder clips (D).

| | Cellularity | Number of Cells Per Field | Cell Preservation | Ruptured Cells | Hemodilution |
|---|---|---|---|---|---|
| Cytospins (automated) | 1.91 | 1.55 | 2.22 | 0.91 | 1.55 |
| Cytospins (manual) | 1.86 | 1.59 | 2.27 | 1.04 | 1.64 |

Table 1. Mean scores for cellularity, number of cells per HPF (960 objective), cell preservation, proportion of ruptured cells, and hemodilution in cytocentrifuge smears prepared with automated and manual centrifugation.