Stability of Miranda´s donkey red blood cell parameters at room and refrigerated temperature and five periods of storage with a ProCyte Dx haematology analyser (IDEXX)

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BACKGROUND

It is advocate that haematological analyses should be executed quickly after sampling[1]. Storage time and temperature are preanalytical factors that are proven to affect the stability of blood samples[2]. This is important in animals like Miranda´s donkey, an autochthonous Portuguese breed that lives in the Miranda plateau, far away from laboratories.[2,3] Delaying analysis can happen in this cases due to shipment of specimens to a central laboratory[2].

Figure 1. Miranda´s Donkey.

Figure 2. ProCyte Dx haematology analyser (IDEXX).

OBJETIVE

To evaluate the stability of Miranda´s donkey red blood cell parameters at room and refrigerated temperature and five periods of storage with ProCyte Dx haematology analyser (IDEXX).

MATERIALS AND METHODS

Eight EDTA-K2 whole blood samples were analyzed, with ProCyte Dx (IDEXX), at room and refrigerated temperature and within 6, 12, 24, 48 and 72 hours of sampling following manufacturer's instructions. The stability of red blood cell parameters was evaluated using one-way analysis of variance (ANOVA) and Tukey's test for comparison of means ($p<0.05$). The study was approved by the ORBEA (Ethics Committee for Animal Welfare) by the Universidade Trás-os-Montes e Alto Douro (UTAD)—i467-e-CECAV-2022 and was carried out in winter.

RESULTS AND DISCUSSION

Influence of storage time was observed at both room and refrigerated temperature. In both temperatures, mean corpuscular volume and mean corpuscular haemoglobin mean´s shown no statistically significant differences during the entire study period ($p>0.05$). At refrigeration temperature, parameters presented some changes, with an increase of the stability limit in hematocrit and mean corpuscular hemoglobin concentration, as well as a decrease of the stability limit in red blood cells count and red cell distribution width. Room temperature hematocrit, along with refrigeration hematocrit and hemoglobin, were the parameters with the lowest stability limit detected (6 hours).

Table 1. Stability data of red blood cell parameters, for eight EDTA-K2 anticoagulated Miranda´s Donkey whole blood samples, at room and refrigerated temperature and five periods of storage with a ProCyte Dx haematology analyser (IDEXX).

Parameters/ Units	0h Mean (95% CI)	6h Mean (95% CI)	12h Mean (95% CI)	24h Mean (95% CI)	48h Mean (95% CI)	72h Mean (95% CI)	Stability limit (h)
			Room Temperature				
RBC (M/µL)	5.20 ^{a} (4.63–5.78)	5.31 ^{a} (4.73–5.88)	6.39 ^{a} (5.82–6.97)	6.00 ^{a} (5.42–6.57)	5.40 ^{a} (4.82–5.97)	5.54 ^{a} (4.96–6.11)	**72**
HCT (%)	29.13 ^{b} (26.71–31.54)	29.35 ^{b} (26.93–31.77)	35.69 ^{b} (33.27–38.10)	33.79 ^{a} (31.37–36.20)	31.25 ^{ab} (28.83–33.67)	33.81 ^{ab} (31.40–36.23)	**6**
HGB (g/dL)	9.80 ^{b} (9.02–10.58)	9.95 ^{b} (9.17–10.73)	12.13 ^{a} (11.34–12.91)	11.28 ^{ab} (10.49–12.06)	10.18 ^{b} (9.39–10.96)	10.41 ^{b} (9.36–11.20)	**6**
MCV (fL)	56.39 (53.36–59.42)	55.70 (52.67–58.73)	56.15 (53.12–59.18)	56.64 (53.61–59.70)	58.31 (55.28–61.35)	61.54 (58.51–64.57)	**72**
MCH (pg)	18.98 (18.06–19.89)	18.89 (17.97–19.80)	19.09 (18.17–20.00)	18.90 (17.98–19.82)	18.98 (18.06–19.89)	18.94 (18.02–19.85)	**72**
MCHC (g/dL)	33.68 ^{a} (32.91–34.44)	33.93 ^{a} (33.16–34.69)	33.99 ^{a} (33.23–34.75)	33.36 ^{a} (32.60–34.12)	32.58 ^{a} (31.81–33.34)	30.84 ^{b} (30.08–31.60)	**48**
RDW (%)	22.96 (22.01–23.92)	23.16 (22.21–24.12)	24.10 (23.15–25.05)	23.64 (22.68–24.59)	23.03 (22.07–23.98)	23.04 (22.08–23.99)	**72**
			Refrigeration (4-6 ºC)				
RBC (M/µL)	5.20 ^{b} (4.45–5.95)	5.35 ^{b} (4.60–6.09)	6.46 ^{ab} (5.71–7.21)	7.65 ^{a} (6.90–8.40)	7.02 ^{a} (6.27 –7.76)	6.18 ^{ab} (5.43–6.93)	**12**
HCT (%)	29.13 ^{d} (25.83–32.42)	29.41 ^{cd} (26.12–32.71)	35.88 ^{abc} (32.58–39.17)	42.89 ^{a} (39.59–46.18)	39.76 ^{ab} (36.47–43.06)	35.00 ^{bcd} (31.70–38.30)	**12**
HGB (g/dL)	9.80 ^{d} (8.70–10.90)	10.06 ^{cd} (8.96–11.17)	12.21 ^{abc} (11.11–13.32)	14.34 ^{a} (13.23–15.44)	13.13 ^{ab} (12.02–14.23)	11.53 ^{bcd} (10.42–12.63)	**6**
MCV (fL)	56.39 (53.38–59.39)	55.45 (52.44–58.46)	55.84 (52.83–58.84)	56.46 (53.46–59.47)	57.06 (54.06–60.07)	57.26 (54.26–60.27)	**72**
MCH (pg)	18.98 (18.11–19.84)	18.95 (18.08–19.82)	19.00 (18.13–19.87)	18.86 (18.00–19.73)	18.81 (17.95–19.68)	18.84 (17.97–19.71)	**72**
MCHC (g/dL)	33.68 ^{a} (33.04–34.31)	34.23 ^{a} (33.59–34.86)	34.06 ^{a} (33.43–34.70)	33.44 ^{a} (32.80–34.07)	33.01 ^{a} (32.38–33.65)	32.90 ^{a} (32.27–33.54)	**72**
RDW (%)	22.96 ^{b} (22.10–23.83)	23.16 ^{b} (22.30–24.03)	24.26 ^{ab} (23.40–25.13)	25.19 ^{a} (24.32–26.05)	25.10 ^{a} (24.23–25.97)	24.78 ^{ab} (23.91–25.64)	**12**

RBC: Red blood cells count; HCT: Hematocrit; HGB: Hemoglobin concentration; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red cell distribution width. Different letters between parameters indicates statistical significance ($p<0.05$).

CONCLUSION

Storage at room and refrigerated temperature must take into account the different stability times and must not exceed 6 hours in both cases. Refrigeration has not been shown to have advantages over storage at room temperature, depending on the parameter. Awareness of this helps in avoiding pre-analytical errors in Miranda´s donkey haematology. Literature is contradictory on this subject and information about donkeys is scarce, so new studies are necessary to better establish parameters stability.

REFERENCES

[1] Zhang, C.; Zhang, H.; Li, G.; Zhang, H. & Fei, Y. (2019). Analysis of time and temperature stability of EDTA anticoagulation whole blood for complete blood count parameters with the use of Abbott cell-Dyn Sapphire Hematology Analyzer. *International Journal of Laboratory Hematology*, 42(2); [2] Perez-Ecija, A.; Buzon-Cuevas, A.; Aguilera-Aguilera, R.; Gonzalez-De Cara, C. A. & Mendoza, F. J. (2020). Blood storage conditions affect hematological analysis in samples from healthy donkeys and donkeys with experimentally-induced endotoxemia. *Frontiers in Veterinary Science*, 7; [3] Silva, G.; Queiroga, F.; Ferreira, M.; Andrade, D. & Silvestre-Ferreira, A.C. (2023). Establishment of Reference Intervals of Hematological Parameters and Evaluation of Sex and Age Effect in the Miranda Donkey. *Animals*, 13, 2331.

Acknowledgements: Members of CECAV received support by the projects UIDB/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). G.M. is supported with a PhD Grant (UI/BD/15084o - FCT).