

BRIEF COMMUNICATION

Hematologic and biochemical reference intervals for adult Friesian horses from North America

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Key Words

Breed-specific, equine, Olympus AU400, Reference Value Advisor, Sysmex XT-2000iV

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DOI:10.1111/vcp.12248

Background: Established breed-specific reference intervals (RI) are an important tool for monitoring the health of horses. There is a lack of published work on breed-specific RI for Friesian horses.

Objectives: The goal of this project was to determine hematologic and biochemical RI for Friesian horses residing in North America.

Methods: Strict inclusion and exclusion criteria were established for selection of reference subjects and for blood specimen collection and handling. Blood samples from 123 healthy, adult (range 3–18 years, median 8 years) Friesian horses of both sexes (70 mares, 45 geldings, and 8 stallions) were used to determine RI. Complete blood counts (CBC) and biochemistry profiles were performed on the Sysmex XT-2000iV hematology and Olympus AU400 biochemistry analyzers, respectively, at IDEXX Laboratories Inc. (Columbus, OH, USA). Results were analyzed using Reference Value Advisor. According to the guidelines of the ASVCP, nonparametric RI with 90% confidence intervals were determined.

Results: IDEXX equine RI are transferrable to Friesian horses for 30 of 36 analytes. Friesian-specific RI (medians) are recommended for the following variables: RBC $5.02\text{--}8.74 \times 10^6/\mu\text{L}$ (6.66), HCT 27–42% (34), HGB 9.0–14.3 g/dL (11.4), lactate dehydrogenase 299–866 U/L (493), direct bilirubin 0.3–0.7 mg/dL (0.5), and anion gap 7–18 mEq/L (12).

Conclusions: The RI established in this study provide a useful baseline for the assessment of hematologic and biochemical data in Friesian horses residing in North America.

The modern Friesian horse has a unique origin and history. Originating in the Friesland region of the Netherlands, its roots go back to the primitive *Equus robustus* (big horse). Initially, Friesians were used as medieval warhorses¹ and later were crossed with Arabian stock, likely Spanish Andalusian horses, as demand shifted toward draft horses. Although the Friesian has retained some of the features of its cold-blooded ancestors, namely their stature, body mass, and calm gentle disposition, it is widely

accepted and thought of as a warm-blooded horse. Older sources present conflicting reports regarding hematologic and biochemical differences in warm-blooded vs cold-blooded equine breeds.² It is unknown which ancestor the Friesian horse may most resemble with regard to blood values. Studies have documented the limited genetic diversity of Friesian horses resulting from declines in breeding populations in the early 20th century.³ This limited gene pool is considered responsible for an increased prevalence of several disorders in Friesian horses⁴, including megaesophagus⁵, hydrocephalus⁶, allergic hypersensitivity⁷, retained placenta³, and dwarfism.⁸ The heritable nature of some of these disorders is supported by several studies.^{3,7} Efforts to decrease the incidence of these disorders through carefully

This study was presented as an abstract by Dr. Katherine M. Fox at the 59th Annual Convention of the American Association of Equine Practitioners, December 7–11, 2013, Nashville, TN.

Dr. Friedrichs, an editor of the journal, was not involved in the peer-review process or the decision to publish this article.

considered breeding are ongoing in the Friesian horse community.

Evaluation of laboratory data is an important component in overall health evaluation, and reference intervals (RI) specific to the Friesian breed will help establish a physiologic database. In addition, breed-specific RI for Friesian horses should minimize errors in clinical decision making. Use of inappropriate RI can lead to both over- and underdiagnosis of disease, either of which can compromise well-being of the horse and increase healthcare costs for the owner. This study was motivated by a failed prepurchase examination based on abnormal blood results in an otherwise healthy Friesian horse. Therefore, the objective of this study was to establish hematologic and biochemical RI for healthy, adult Friesian horses in North America using a commercial reference laboratory. It was hypothesized that some hematologic and biochemical values will be different for Friesian horses compared to the general horse population.

This study followed the ASVCP guidelines for establishing RI in veterinary species and was conducted from April through November, 2012.⁹ Inclusion and exclusion criteria were established prior to sample collection. The reference population included healthy, adult Friesian horses of both sexes residing in the United States and Canada. Adults were defined as horses between the ages of 3 and 20 years, and registration papers were used to verify age. Each owner was provided a questionnaire for recording the horses' use, husbandry, and pertinent medical history. Health was defined as no evidence of disease on the day of or in the 30 days preceding and following blood collection; the latter was verified by follow-up communication (KMF). A complete physical examination was performed by a veterinarian at the time of blood collection to establish absence of disease. All horses were required to have a current negative test for equine infectious anemia.

Horses were excluded if they were deemed unhealthy, had been vaccinated in the preceding 30 days, received medications other than routine antiparasitics, or were pregnant or lactating mares. Horses that displayed excitement or agitation at the time of sample collection, or had undergone strenuous exercise in the preceding 48 hours were also excluded. Blood specimens were excluded if there were clots detected in the EDTA tube at processing, if there was greater than mild hemolysis, icterus, or lipemia in the serum sample, or if sample collection and handling protocols were not followed. Finally, horses were excluded if subsequent examination of blood results led to a suspicion of underlying disease.

Horses were fasted 4 hours prior to blood collection. A veterinarian or veterinary technician collected blood samples from the jugular vein directly into a 10 mL vacutainer serum separator tube and a 3 mL EDTA vacutainer tube (Becton Dickinson and Co, Franklin Lakes, NJ, USA) utilizing a 20 ga × 1.5" blood collection needle. The serum separator tube was allowed to clot for one hour prior to centrifugation; however, in a few cases, a delay up to 5 hours may have occurred. Centrifugation was performed at the local veterinary practice following routine protocols. Both tubes were refrigerated (4°C) prior to placement in laboratory-provided shippers containing ice packs and shipped overnight to the reference laboratory for processing.

All samples were received and processed by the reference laboratory (IDEXX Laboratories Inc., Columbus, OH, USA) within 24 hours of collection. Two blood smears were made and microscopically examined for platelet clumps and visual verification of automated leukocyte differential counts. The CBC was performed on a Sysmex XT-2000iV hematology analyzer (Sysmex Corporation, Kobe, Japan) and the following variables were reported: WBC and RBC count, HGB, HCT, MCV, MCH, MCHC, platelet concentration (PLT), and automated absolute counts for neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Manual differential leukocyte counts were reported if microscopic examination did not verify the automated count. Fibrinogen was measured by the heat precipitation method. Biochemical profiles were performed on an Olympus AU400 chemistry analyzer (Olympus America Inc., Melville, NY, USA) and the following variables were reported: ALP, AST, CK, GGT, and lactate dehydrogenase (LDH) activities, and concentrations of albumin (ALB), total protein (TP), total bilirubin (TBILI), direct bilirubin (DBILI), urea, creatinine (CREA), cholesterol (CHOL), glucose (GLU), calcium (Ca), phosphorus (PHOS), total CO₂ (TCO₂), chloride (Cl), potassium (K), and sodium (Na). The following variables were calculated: globulin (GLOB = TP - ALB) concentration, indirect bilirubin (INBILI = TBILI - DBILI) concentration, and anion gap (AG = [Na + K] - [Cl + TCO₂]). Thyroxine was analyzed on the same chemistry analyzer using commercial reagents (Microgenics, Fremont, CA, USA). Quality control procedures were performed every 12 hours with commercial QC materials, and samples were run only when the control runs passed inspection.

Reference intervals were determined according to the guidelines of the ASVCP⁹ and analyzed using Reference Value Advisor.¹⁰ Histograms were examined

for estimation of data distribution and to identify the presence of outliers. Distribution was further assessed by Anderson–Darling normality test with a $P < .05$ indicating the data were significantly different from a Gaussian distribution. Outliers were detected using Dixon's range statistic ($r > .3$). For PLT, outliers were detected using Tukey's interquartile fences after transformation (Box–Cox) to Gaussian distribution. Upper (97.5th percentile) and lower (2.5th percentile) limits were established nonparametrically and 90% confidence intervals (CI) were determined nonparametrically around these limits. Total number of samples suitable for platelet RI was < 120 ; therefore, robust methods were used to calculate the reference limits.⁹

Guidelines for transference of RI were used to determine whether IDEXX equine RI (IDEXX equine RI reported during the dates of this study, April through November 2012) are appropriate for use in Friesian horses.⁹ Based on the number of samples collected (≥ 120 samples), the following criteria were used: if ≤ 12 of 120 Friesian blood results fell outside the equine RI, it was acceptable for use in Friesian horses, and if ≥ 13 of 120 fell outside the RI, they were unacceptable. This method is analogous to a binomial analysis.¹¹

Blood was collected from 129 horses between April and November of 2012. One sample was excluded due to improper handling. Two horses were excluded because of poor sample quality (marked hemolysis). Three horses did not meet physiologic criteria (hyperfibrinogenemia, azotemia, lactation) and were excluded. The remaining 123 horses consisted of 70 mares, 45 geldings, and 8 stallions. Ages ranged from 3–16 years with a median age of 8 years (Figure 1). Horses resided in 21 states including Wisconsin ($n = 40$), Washington ($n = 16$), Ohio ($n = 11$), California ($n = 8$), Maryland ($n = 8$), Idaho ($n = 6$), Michigan ($n = 4$), New York ($n = 4$), Texas ($n = 4$), Minnesota ($n = 3$), New Mexico ($n = 3$), Montana ($n = 2$), Georgia ($n = 2$), Massachusetts ($n = 2$), Oklahoma ($n = 2$), Arkansas ($n = 1$), Colorado ($n = 1$), Illinois ($n = 1$), Kentucky ($n = 1$), New Jersey ($n = 1$), Tennessee ($n = 1$), and Canada ($n = 2$). Fifty-seven horses were used for pleasure riding or light work, 18 were broodmares, 12 were used for competition or moderate work, and 36 were idle. Management, housing, nutrition, general husbandry, vaccination, and deworming protocols met the inclusion criteria.

Hematologic and biochemical RI are reported in Tables 1 and 2, respectively. Seven of 36 analytes had Gaussian distributions. An additional analyte had a Gaussian distribution after one outlier was eliminated

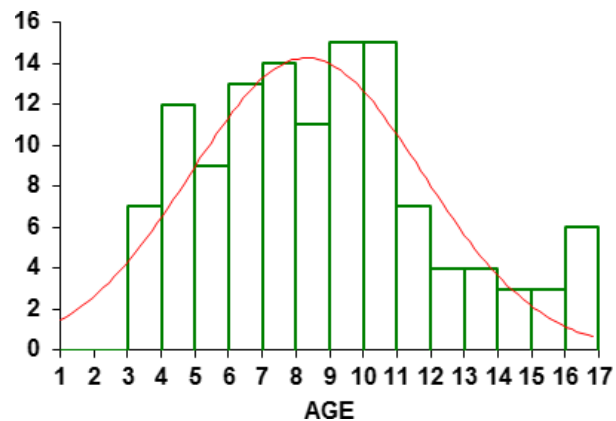


Figure 1. Age distribution of 123 adult Friesian horses.

(Ca). A single high outlier was identified by the Dixon's range test and eliminated for concentrations of INBILI, Ca, and K, and 2 outliers were identified and eliminated for absolute basophil count. Two results for CK and 2 for LDH activities in 4 different horses appeared to be outliers based on the histograms, but these results were not identified as outliers by the Dixon's range test and thus were included in the analysis. Platelet counts were excluded on 13 samples because of either clumping ($n = 9$) or a numerical value was not reported ($n = 4$). Automated differential leukocyte counts were reported for all but one horse for which a manual differential leukocyte count was reported. During the statistical analysis phase of the study, it was learned that serum separation may have been delayed for up to 5 hours in a few unidentified samples. Because delayed separation will decrease glucose concentration, results are not included in Table 2.

Based on the rules for transference, IDEXX equine RI were rejected for 3 hematologic (RBC count, HGB, HCT) variables and 3 biochemical (DBILI, LDH, AG) analytes. Eleven hematologic and 19 biochemical RI all fell within IDEXX general equine RI (Tables 1 and 2). The hematologic RI for RBC count, HGB, and HCT trended lower for the Friesian horse breed with 51, 40, and 34 horses having values below the IDEXX equine RI, respectively. The biochemical analytes DBILI concentration and LDH activity trended higher, with 43 and 81 horses having values above the IDEXX RI, respectively. AG had a wider interval with 9 horses having values below the IDEXX RI and 10 horses with values above the IDEXX RI.

Not addressed by the transference method of RI suitability are variables for which no results fell outside IDEXX equine RI or for variables that have upper or lower limits that differ from the reference laboratory's limits. Among these, 6 biochemical (ALB, TP, CHOL,

Table 1. Nonparametric hematologic reference intervals (RI) for healthy adult Friesian horses.

Hematologic Variable	Descriptive Statistics				RI within 90% CI			n	Distribution	IDEXX RI*
	Mean	Med	SD	Min/Max	RI	Lower Limit	Upper Limit			
WBC (10 ³ /μL)	7.6	7.6	1.5	5.1–11.6	5.3–10.9	5.1–5.5	10.1–11.6	123	G	4.6–11.4
RBC†(10 ⁶ /μL)	6.70	6.66	0.85	4.96–9.51	5.02–8.74	4.96–5.46	8.09–9.51	123	G	6.5–12.5
HGB† (g/dL)	11.5	11.4	1.3	8.5–16.8	9.0–14.3	8.5–9.6	13.8–16.8	123	NG	11–19
HCT† (%)	34	34	3.7	24–48	27–42	24–28	40–48	123	G	32–52
MCV (fL)	51	51	3	41–61	45–58	41–45	57–61	123	NG	34–58
MCH (pg)	17.1	17.3	0.9	14.9–19.2	15.3–19.1	14.9–15.4	18.6–19.2	123	G	12.3–19.7
MCHC (g/dL)	34	34	1	31–37	32–36	31–32	36–37	123	NG	31–37
PLT (10 ³ /μL)	152	153	42	67–274	90–245	73–87	229–261	110	NG	100–350
NEU (cells/μL)	3816	3696	793	1976–6540	2558–5615	1976–2742	5175–6540	123	NG	2260–8580
LYM (cells/μL)	3174	3003	1017	1265–6310	1541–5300	1265–1690	4948–6310	123	NG	1500–5000
MONO (cells/μL)	329	325	102	105–728	174–580	105–192	486–728	123	NG	0–1000
EOS (cells/μL)	243	188	172	9–820	59–726	9–68	630–820	123	NG	0–1000
BASO (cells/μL)	32	23	34	0–128	0–104	0–0	93–128	121	NG	0–29
FIB (g/dL)	0.3	0.2	0.7	0.1–0.4	0.1–0.4	0.1–0.2	0.4–0.4	123	NG	0.1–0.4

CI indicates confidence interval; MIN, minimum; MAX, maximum; n, number of samples; NG, non-Gaussian distribution; G, Gaussian distribution; NEU, absolute neutrophil count; LYM, absolute lymphocyte count; MONO, absolute monocyte count; ESO, absolute eosinophil count; BASO, absolute basophil count.

*IDEXX equine RI reported during the dates of this study, April–November, 2012.

†Indicates nontransferable reference interval.

Table 2. Nonparametric biochemical reference intervals (RI) for healthy adult Friesian horses.

Biochemical Variable	Descriptive Statistics				RI with 90% CI			n	Distribution	IDEXX RI*
	Mean	Med	SD	Min/Max	RI	Lower Limit	Upper Limit			
ALP (U/L)	130	123	37	58–228	65–222	58–79	206–228	123	NG	73–327
AST (U/L)	331	325	53	232–537	236–474	232–258	414–537	123	NG	168–408
CK (U/L)	259	208	154	111–1128	117–741	111–129	495–1128	123	NG	110–700
GGT (U/L)	18	16	6	7–41	10–37	7–10	29–41	123	NG	5–35
ALB (g/dL)	3.3	3.3	0.2	2.8–3.9	2.8–3.8	2.8–3.0	3.7–3.9	123	NG	2.6–4.2
TP (g/dL)	6.5	6.4	0.4	5.5–7.6	5.7–7.4	5.5–5.9	7.2–7.6	123	NG	5.4–7.6
GLOB (g/dL)	3.1	3.1	0.4	2.3–4.4	2.4–3.9	2.3–2.5	3.8–4.4	123	G	1.8–4.3
TBILI (mg/dL)	0.9	0.8	0.3	0.4–1.9	0.5–1.4	0.4–0.5	1.3–1.9	123	NG	0.6–3.7
DBILI† (mg/dL)	0.5	0.5	0.1	0.3–0.8	0.3–0.7†	0.3–0.3	0.7–0.8	123	NG	0–0.5
UREA (mg/dL)	16	17	3.7	5–28	8–23	5–10	22–28	123	NG	10–27
CREA (mg/dL)	1.1	1.1	0.2	0.7–1.6	0.8–1.6	0.7–0.8	1.5–1.6	123	NG	0.8–2.2
CHOL (mg/dL)	93	92	14	70–131	70–127	70–74	119–131	123	G	49–150
Ca (mg/dL)	11.4	11.4	0.49	10.3–12.7	10.5–12.5	10.3–10.6	12.3–12.7	122	G	10.5–12.8
PHOS (mg/dL)	3.5	3.4	0.6	2.3–5.5	2.4–5.0	2.3–2.8	4.7–5.5	123	NG	1.4–5.6
TCO ₂ (mEq/L)	28	28	2	22–35	23–32	22–25	31–35	123	NG	25–34
Cl (mEq/L)	100	100	2	93–1.7	95–105	93–96	104–107	123	NG	91–105
K (mEq/L)	4	4	0.35	3–5	3.3–4.9	3–3.3	4.6–5.0	122	NG	3–5.3
Na (mEq/L)	136	136	2	131–141	132–139	131–133	139–141	123	NG	130–140
INBILI (mEq/L)	0.4	0.3	0.14	0.1–0.8	0.1–0.7	0.1–0.2	0.7–0.8	122	NG	0–3.0
AG† (mEq/L)	12	12	3	4–21	7–18†	4–8	17–21	123	NG	9–16
LDH† (U/L)	512	493	144	255–1070	299–866†	255–313	751–1070	123	NG	140–440
Thyroxine (μg/dL)	1.7	1.6	0.4	0.6–2.9	0.7–2.6	0.6–0.9	2.4–2.9	123	G	1.0–3.8

CI indicates confidence interval; MIN, minimum; MAX, maximum; n, number of samples; ALB, albumin; TP, Total protein; GLOB, calculated globulin; TBILI, total bilirubin; DBILI, direct bilirubin; CREA, creatinine; CHOL, cholesterol; Ca, calcium; PHOS, phosphorus; TCO₂, total carbon dioxide; Cl, chloride; K, potassium; Na, sodium; IBILI, calculated indirect bilirubin; AG, anion gap; LDH, lactate dehydrogenase; NG, non-Gaussian distribution; G, Gaussian distribution.

*IDEXX equine RI reported during the dates of this study, April–November, 2012.

†Indicates nontransferable reference interval.

INBILL, K, PHOS) and 3 hematologic variables (MCH, absolute monocyte and eosinophil count) had zero Friesian results falling outside IDEXX equine RI. For these variables, one or more of the limits may be too wide for Friesian horses, resulting in a failure to recognize atypical results in Friesian horses and possible underdiagnosis.

This study was specifically designed for the field practitioner by utilizing a large, nationally established reference laboratory easily accessible to North American equine practitioners. The breed or type of horses used to establish IDEXX equine RI is not available nor are RI available based on breed type. This can be true for many RI used by veterinarians. Few published studies report breed or type-specific RI in horses^{12,13}, and older studies use obsolete analyzers^{2,14} so that comparisons to current studies cannot be made. Without further studies, differences in breed-specific RI and the applicability of general RI to specific horse breeds are unknown. Establishing and utilizing breed-specific RI for all equine breeds would be challenging for the laboratory and veterinarian alike, but knowledge of differences in certain analytes for breed types (cold-blooded vs warm-blooded) is essential to clinical interpretation of blood values.

Lactate dehydrogenase activity was included in this study because it is commonly utilized by equine practitioners. The Friesian breed trended toward higher LDH activities. This trend was also reported in the Spanish Andalusian breed¹², a potentially interesting finding considering the history of the Friesian breed. However, a higher RI for CK and AST activities in Andalusians were speculated to be related to the handling of the horses prior to sampling. In our sample population, care was taken to avoid excitement during collection and recent strenuous exercise, and samples with moderate to marked hemolysis were excluded. Because AST and CK activities were similar to IDEXX equine RI, it is unlikely that hepatocellular or muscular injury is the cause of higher LDH activity. In addition, Friesian horses in this study were carefully screened for disease with a thorough history, physical examination, and 30-day follow-up on the health status of the reference subjects; therefore, it is unlikely that the variables that frequently fell outside the IDEXX equine RI indicated the presence of underlying disease. Therefore, it is speculated that the higher LDH activities seen in North American Friesians represent a breed-specific trait.

Automated leukocyte differential counts were reported for all but one horse in this study. Automated differential leukocyte counts were verified by microscopic examination of blood smears, and all Sysmex

cytograms showed good separation of leukocyte types. In one horse for which a manual count was reported, the automated and manual counts were not substantially different. Because the purpose of this study was to determine RI using an accessible reference laboratory, standard protocols regarding the reporting of leukocyte differential counts were followed. In the absence of pathologic changes in leukocyte morphology, distinct separation of equine leukocyte types is readily accomplished by the Sysmex XT-2000iV hematology analyzer facilitating automated differential leukocyte counts.¹⁵ However, microscopic examination of blood smears is recommended to verify automated leukocyte differential counts, especially when leukocyte types are not distinctly separated or demonstrate abnormalities on cytograms. Reporting of morphologic abnormalities in all blood cells is an essential component of a CBC.

Hematologic and biochemical variables can be influenced by many parameters ranging from signalment, health, husbandry, and ontogenic traits to the specific analyzer and methodology used to generate results.⁹ This study attempted to limit these parameters by evaluating adult Friesian horses with documented husbandry and health histories, and utilizing a nationally accessible reference laboratory. As a result, these RI should provide a reliable baseline for adult Friesian horses residing in North America when specimens are analyzed using the same analyzers and methods. Although general IDEXX equine RI were suitable for interpretation of many variables, Friesian-specific RI are recommended for RBC count, HCT, HGB, LDH activity, AG, and DBILI concentration to avoid misinterpretation of blood results. Future studies may include age-specific and gender-specific RI.

Acknowledgments

This work was made possible through the financial support of the Fenway Foundation for Friesian Horses and IDEXX Laboratories Inc., Columbus, OH.

Disclosure: Two of the authors (DW and ER) are employees of IDEXX Laboratories Inc.

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