Clinical Pathology Standard Parameters in Nude Rats: *De Novo* Generation Of Reference Intervals

TOX AND DIAG
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Introduction

Long term pharmacology and safety evaluation of innovative human-derived cell therapy products require the use of immunocompromised animals to avoid immune-mediated destruction of injected human-derived cells. The Athymic Nude (RH-*FoxN1*^{rnu}) rat is a T-cell- deficient model used in preclinical research for various applications (xenograft research, tumor biology, immunology). Athymic Nude rats represent an interesting alternative to broadly used Nude mice, particularly when implantation surgery is required, and animals of larger size are preferred.

In preclinical research, control animals may not be included into the study protocol and in specific cases population-based reference intervals may be needed for data interpretation for example in assessing health status of animals. Clinical pathology data obtained from this rat strain using the same instruments as ours remain absent in the literature.

The aim of this study is to provide population-based reference intervals for RH-*FoxN1*^{rnu} Nude rats for standard hematology, biochemistry and coagulation parameters.

Materials and Methods

A total of 34 clinically healthy and untreated RH-*FoxN1*^{rnu} Nude rats (16 males and 18 females) provided by *Envigo RMS*, Netherlands were included in this prospective study. Females were nulliparous and non-pregnant. Animals were aged 9.5 months at the time of sampling. They were housed at ERBC Baugy facilities for 8 months, by gender, up to 3 individuals per cage in standard-sized cages.

Blood samples were collected on anesthetized animals (medetomidine premedication followed by isoflurane anesthesia) at the abdominal aorta. Blood was anticoagulated for analyses with lithium heparin for biochemistry, citrate for coagulation and K3 EDTA for hematology.

Standard biochemistry, coagulation and hematology data were obtained from all animals following internal procedures on validated analyzers (Pentra C400, STA-Satellite Max and Sysmex XN-1000V, respectively). Population-based reference intervals were generated following the ASVCP reference interval guidelines.
Individual data were reviewed to detect possible pre-analytical or analytical errors. Outliers were detected using statistical methods (Dixon's range statistic and Tukey's interquartile fences). Given the small sample size (n<40), reference intervals were generated by the robust or standard method depending on the nature of the distribution of innate or box-Cox transformed data. Statistical analyses and reference interval determination were done using Reference Value Advisor V2.1.

Results

Two hematology samples and one citrated sample were coagulated and therefore not analyzed. In rare instances, for isolated parameters, values were not obtained (insufficient volume for instance).

For each parameter, the number of values (N), minimum and maximum values and calculated reference intervals are presented in Table 1.

An illustrative example of hematology scatter plots obtained from the Sysmex XN-1000V hematology analyzer is presented in Figure 1.

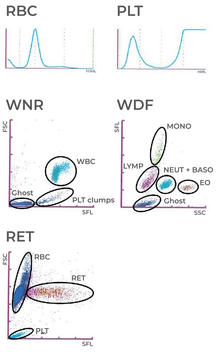
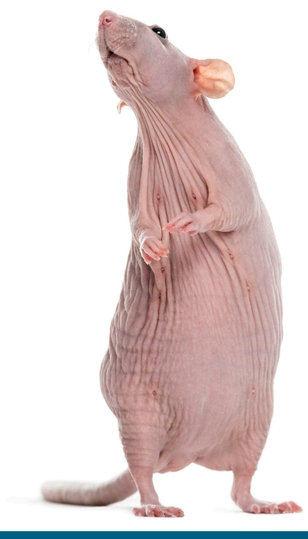


Figure 1. Example of hematology graphs obtained with the Sysmex XN-1000V analyzer

Clockwise from top left: red blood cell volume histogram, platelet volume histogram, scatterplot of the WDF channel, reticulocyte scatterplot, and scatterplot of the WNR channel, obtained from one of the study animals after blood analysis using the Sysmex XN-1000V analyser.

FSC	forward-scattered light
SSC	side-scattered light
SFL	side-fluorescence light
LYMP	lymphocytes
MONO	monocytes
NEUT	neutrophils
BASO	basophils
EO	eosinophils
Ghost	red blood cell ghosts
WBC	white blood cells
PLT	platelets
RBC	red blood cells
RET	reticulocytes



Parameter	Unit	N	Min-Max	Innate distribution	Method	Reference interval
Biochemistry						
Sodium	mmol/L	34	131-139	NG	RT	132.6-138.5
Potassium	mmol/L	34	3.07-6.02	NG	ST	3.1-5.6
Chlorure	mmol/L	34	101.1-109.4	G	S	101.8-108.7
Calcium	mmol/L	33	2.5-2.88	G	S	2.5-2.8
Phosphorus	mmol/L	33	1.33-3.34	G	S	1.3-3.0
Urea	mmol/L	34	4.4-9.5	G	S	3.6-8.3
Creatinine	µmol/L	34	28-46	G	S	27.3-45.4
AST	UI/L	32[a]	26-133	NG	ST	34.3-109.3
ALP	UI/L	34	26-120	NG	ST	25.9-126.2
ALT	UI/L	33[b]	15-51	G	S	18.1-49.6
Total bilirubin	µmol/L	29[c]	1.17-2.41	NG	ST	1.1-2.5
Glucose	mmol/L	33	10.75-30.72	G	S	9.4-31.4
Triglycerides	mmol/L	33[d]	0.26-2.21	NG	ST	0.3-2.2
Cholesterol	mmol/L	34	1.56-3.38	G	S	1.4-3.0
Total proteins	g/L	33[e]	45-70	NG	RT	48.7-66.9
Albumin	g/L	33	20-36	NG	RT	22.2-33.1
Globulins	g/L	33	25-38	G	S	24.3-37.4
Coagulation						
aPTT	s	30[f]	14.9-24.2	NG	ST	12.6-24.2
PT	s	31	16.5-25.1	G	S	16.8-25.9
Fibrinogen	g/L	31	1.01-4.46	G	S	0.7-4.3
Hematology						
Hematocrit	%	31[g]	34.8-41.8	G	S	35.3-42.7
Red Blood Cells	10^{12}/L	31[h]	6.57-9.12	G	S	6.6-8.9
Hemoglobin	g/dL	31[i]	12.7-14.8	G	S	12.6-15.0
Reticulocytes	10^9/L	31[p]	157.8-333.8	G	S	136.9-285.9
MCV	fL	32	42.1-58.3	NG	RT	44.5-55.7
MCHC	g/dL	31[j]	33.5-37.1	G	S	33.8-37.1
MCH	pg	31[k]	14.6-20.7	NG	RT	15.7-19.9
RDW	%	32	10.5-23.8	NG	ST	10.3-23.8
WBC	10^9/L	29[l]	1.7-15.67	NG	RT	1.6-14.2
Neutrophils	10^9/L	29[m]	0.74-8.21	NG	ST	0.7-8.1
Lymphocytes	10^9/L	32	0.5-3.6	G	S	0.1-3.4
Monocytes	10^9/L	32	0.2-2.76	NG	ST	0.2-2.9
Eosinophils	10^9/L	30[n]	0.05-0.59	NG	ST	0.1-0.5
Platelets	10^9/L	31	827-1698	G	S	788.0-1536.9
MPV	fL	32	7.4-8.8	G	S	7.6-8.7

Table 1. Biochemistry, coagulation and hematology results and calculated reference intervals.

G: Gaussian, NG: Non-Gaussian, S: Standard, R: Robust, ST: Standard Transformed, RT: Robust Transformed. Eliminated outliers: [a]150 UI/L and 440 UI/L [b]68 UI/L [c]37 µmol/L [d]4.54 mmol/L [e]77 g/L [f]35.8 s [g]19.5 % [h]4.63 10^{12}/L [i]6 g/dL [j]30.8 g/dL [k]13 pg [l]24.91x10^9/L , 15.67x10^9/L and 14.41x10^9/L [m]8.92x10^9/L,10.68x10^9/L and 18.3x10^9/L [n] 0.7x10^9/L and 0.99x10^9/L [o]0.11x10^9/L [p]688.5x10^9/L.
AST: aspartate aminotransferase, ALP: alkaline phosphatase, ALT: alanine aminotransferase, aPTT: activated partial thromboplastin time, PT: prothrombin time, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, MCH: mean corpuscular hemoglobin, RDW : red cell distribution width, WBC: white blood cells, MPV: mean platelet volume.

Discussion and Conclusion

Full clinical pathology profiles were established to characterize the RH-*FoxN1*^{rnu} Nude rat strain and to produce *de novo* reference intervals.

The main limitation of this study was the low number of animals included.

Some outlier values were excluded from our study. A full individual histopathologic assessment is in progress and may lead to reconsideration of animal inclusion into our data set. These data will be published in future works.

Specific hematology changes were observed when compared with data obtained from Sprague Dawley rats in our laboratory using the same hematology analyzer. By qualitative assessment, these changes consisted of lower absolute white blood cell counts due to lower lymphocyte counts. These observations were consistent with the T-cell deficient phenotype of the Nude rat strain. All other biochemistry, coagulation and hematology parameters were overall consistent with values obtained in Spraque-Dawley rats. Further statistical analyses would be required to confirm and bring scientific robustness to these observations.
Overall, the data presented in this poster will be of interest when evaluating clinical pathology data of RH-*FoxN1*^{rnu} Nude rats.

References

▸ The rnu (Rowett Nude) and rnuN (nznu, New Zealand Nude) rat : an update. Schuurman and al. ILAR news, Volume 34, Issue 1-2, 1992, Pages 3-12

▸ ASVCP reference interval guidelnes : determination of *de novo* reference intervals in veterinary species and other related topics. Veterinary Clinical Pathology, Volume 41, Issue 4, December 2012, Pages 441-453

Acknowledgments

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