CHAPTER 3

ANAEMIA IN EAST AFRICAN SHORT-HORN ZEBU CALVES: EVALUATION OF THE FIELD PERFORMANCE OF FAMACHA© COLOUR CHART IN DETECTING ANAEMIA AND THE PERFORMANCE OF THE SYSMEX© AUTOMATED ANALYZER FOR LABORATORY DIAGNOSIS OF ANAEMIA

1. INTRODUCTION

Anaemia is a common clinical sign of many of the tick-borne diseases, trypanosomosis and helminthosis. Anaemia is defined as an erythrocyte count, haemoglobin concentration or a packed cell volume that is below what is considered as reference values for the species (Jain 1993).

Clinical diagnosis of anaemia in the field can be based on the presence of pale mucous membranes. The FAMACHA© eye colour chart was developed for use in small-stock farming in South Africa where helminthosis, in particular wireworm *Haemonchus contortus*, is a major cause of production losses (Anon. 2002b). The system is based on the assessment of colour changes of the mucous membranes as an indication of the development of anaemia in small stock infected with wireworm. The animals are classified into five categories by comparing the ocular mucous membrane with a laminated colour chart with categories ranging from red to pink to white, which represent increasing levels in severity of anaemia. The FAMACHA© has been tested in various countries and is considered a cheap pen-side test, that is easy to learn even by illiterate individuals, and can be a valuable tool in an integrated worm-control programme in sheep (Anon. 2002b; Kaplan, Burke, Terrill, Miller, Getz, Mobini, Valencia, Williams, Williamson, Larsen & Vatta 2004; Depner, Gavi, Cecim, Rocha & Molento 2007) and goats (Kaplan *et al*. 2004; Ejlertsen, Githigia, Otieno, & Thamsborg 2006; Scheuerle, Mahling, Muntwyler & Pfister 2010). This test has also been evaluated in West Africa as a possible tool in the diagnosis and control of trypanosomosis in cattle based on the presence of pale mucous membranes in infected animals (Grace, Himstedt, Sidibe, Randolph & Clausen 2007). The performance of the FAMACHA© eye colour chart as a field diagnostic tool in detecting anaemia in East African short-horn Zebu cattle was evaluated in this study.

The clinical diagnosis of anaemia is subjective and it is also difficult to detect the presence of subclinical cases. Therefore, a clinical diagnosis of anaemia needs to be confirmed by laboratory diagnosis. This is done by measuring the PCV or HCT, HGB and RCC of the animal. Automated cell analyzers such as the Sysmex© poch-100iV (Sysmex South Africa) allow for accurate measurements of various blood cell components in a short time. The performance of the Sysmex analyzer was assessed prior to its use in the field laboratory for this study.

2. MATERIALS AND METHODS

*General methodology (description of study site and sampling population) is discussed in Chapter 2.

2.1 Evaluation of the field performance of the FAMACHA© eye colour chart

The relationship between FAMACHA© score and PCV (%) was initially assessed using all observations of all the calves. Packed cell volume (%) was used as the reference test for anaemia, with PCV<25% considered as anaemic. The effect of age on the field performance of the FAMACHA© test was assessed by comparing the linear relationship between FAMACHA© and PCV (%) for three age groups: 6, 21 and 51 weeks. To exclude the possible effect of repeated measures of the calves as well as the effect of age, the field performance of the FAMACHA© test was then assessed using only data from the 21-week age group. The field performance of the test was measured by the sensitivity (Se) and specificity (Sp), positive (PV+) and negative predictive values (PV-), likelihood ratios (LR+/-) and receiver operating characteristic curves (ROC). A positive likelihood ratio is the odds that an animal is truly diseased when tested as positive. Receiver operating characteristic curves are a measure of accuracy of the test, given the anaemic status of the calf as either positive (PCV<25%) or negative (PCV≥25%). The area under the curve (AUC) of the ROC is an indication of what the probability is that a randomly selected animal with a PCV<25% had a higher FAMACHA© score than a randomly selected animal with a PCV≥25%. An AUC=0.5 can be considered as non-informative, $0.5 < AUC \leq 0.7$ as less accurate, $0.7 < AUC \leq 0.9$ as moderately accurate, $0.9 < AUC \leq 1$ as highly accurate, and AUC = 1 as a perfectly accurate test (Greiner *et al*. 2000). The accuracy of the FAMACHA© test was also investigated with a two-graph ROC plot method (Greiner *et al*. 2000; Reynecke, Van Wyk, Gummow, Dorny & Boomker 2011). On the two-graph ROC plot method the Se and Sp are plotted individually as a function of the FAMACHA© cut-points. To exclude the effect of age on the PCV, only observations at 21 weeks of age were used in the assessment of test performance using different cut-points.

Inter-rater performance of the FAMACHA© eye colour chart (using a FAMACHA© cut-off of 4) was also measured by the Se, Sp, PV+, PV- and ROC. The effect of rater experience on the accuracy of the test was measured by comparing the performance of raters from the first half of the study [Feb 2008 (when the first calves were 21 weeks old) – Sept 2009] to the second half of the study period [Oct 2009 – May 2010 (when the last calves were 21 weeks old)], also only using data from the 21-week visits. Raters scored the subjects only once per visit; therefore no intra-rater performance could be measured.

2.2 Evaluation of the performance of the Sysmex

The laboratory performance of the Sysmex poch-100iV Diff automated analyzer was evaluated before it was used under field laboratory conditions. For this purpose whole blood, using EDTA vacutainer tubes, from 78 clinically healthy bovines was analyzed at the Clinical Pathology laboratory, Onderstepoort Veterinary Academic Hospital, Faculty of Veterinary Science, University of Pretoria. The performance of the Sysmex was compared to an established automated analyzer, the Cell-Dyn® 3700 (Abbott, South Africa) that hasbeen shown to have adequate accuracy and precision with bovine samples. The Sysmex performance under field conditions was also assessed by a comparison between manual PCV and HCT as measured by the Sysmex. The samples from the IDEAL calves were used for this purpose.

The precision reported for the haemogram parameters for the Cell-Dyn®, given as coefficient of variation (CV) with a 95% confidence limit, were as follows: for white blood cell count (WCC) the CV≤2.5%; for red cell counts (RCC) the CV≤2.8%; for haemoglobin concentration (HGB) the CV≤1.2%; for mean corpuscular volume (MCV) the CV≤1.0%; and for platelet counts (Plt) the CV≤5.0%. The precision for the lymphocyte counts (%) was given as a ±2.6 difference from the mean of determinants with a 95% confidence limit (Anon. 2000a). The accuracy of haemogram parameters for the Cell-Dyn®, reported as correlation coefficients (CC) for WCC was CC≥0.99; for RCC the CC≥0.98; for HGB the CC≥0.98; for MCV the CC≥0.98; for Plt the CC≥0.98; and for lymphocyte counts (%) the CC≥0.94 (Anon. 2000a).

Limits of agreement plots (Bland & Altman 1986) were used to indicate the range of differences between the results of the Sysmex and the Cell-Dyn®, and the Sysmex HCT and manual PCV. In these graphs the difference (Δ) between values for each sample was plotted against the average of the values of the two test methods. Intraclass correlations (ICC) were used to assess what proportion of the total variance was accounted for by within-test (test

method, e.g. Sysmex) variation. An ICC close to 1 indicated that within group (test method) variation was small relative to variation between the two test methods.

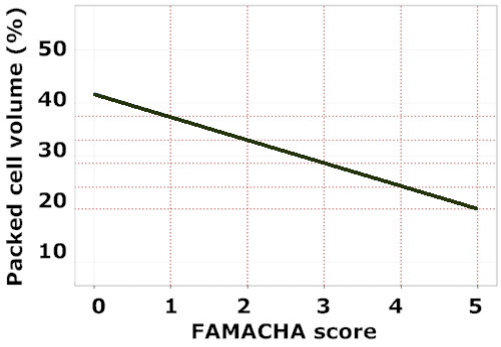
3. RESULTS

3.1 Evaluation of the field performance of the FAMACHA©

3.1.1 The relationship between FAMACHA© and PCV

All observations (n=5637) of all the calves were initially used to analyse the relation between FAMACHA© score and PCV (Fig. 3.1). There was linear relationship between the FAMACHA© score and PCV, with a mean PCV of 37.5%, 33%, 28.6%, 24.1%, and 20% for FAMACHA© score of 1, 2, 3, 4 and 5 respectively.

Figure 3.1 The relation between FAMACHA© score and average PCV (%) (n=5637)



3.1.2 The distribution of FAMACHA© at different age-groups

The distribution of PCV (%) for three age groups (6 weeks, 21 weeks and 51 weeks) is illustrated in Figure 3.2. There were marked differences between the 6-week age group compared to the other two groups, with a mean PCV = 36.12% for the 6-week age group (n=496), mean PCV = 28.94% for the 21-week age group (n=485), and mean PCV = 26.95% for the 51-week age group (n=453).

The frequency distributions of FAMACHA© scores for the three age groups are illustrated in Fig. 3.3a-c. Most calves had a FAMACHA© score of 2 at 6 weeks of age, whereas the majority of calves had a FAMACHA© score of 3 at both 21 and 51 weeks.

Figure 3.2 The distribution of PCV (%) for age groups 6, 21 and 51 weeks

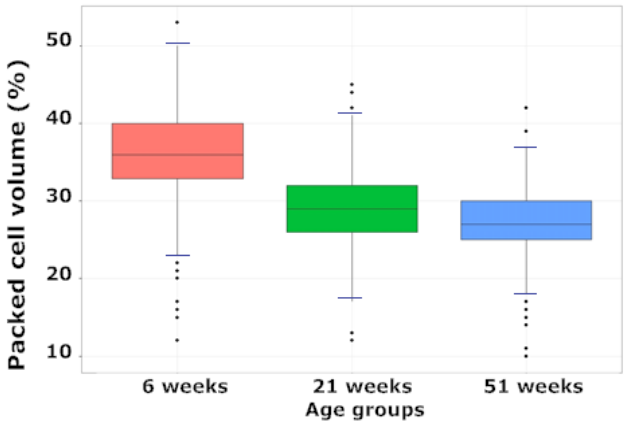


Figure 3.3a The frequency distribution of FAMACHA© scores for the 6-weeks age groups

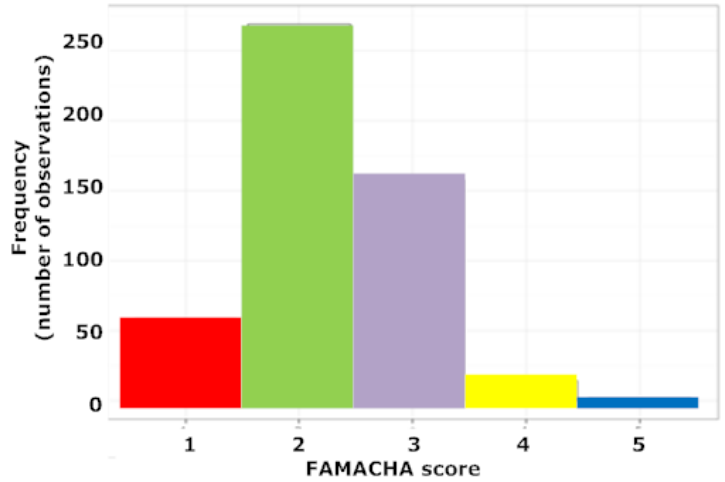


Figure 3.3b The frequency distribution of FAMACHA© scores for the 21-weeks age groups

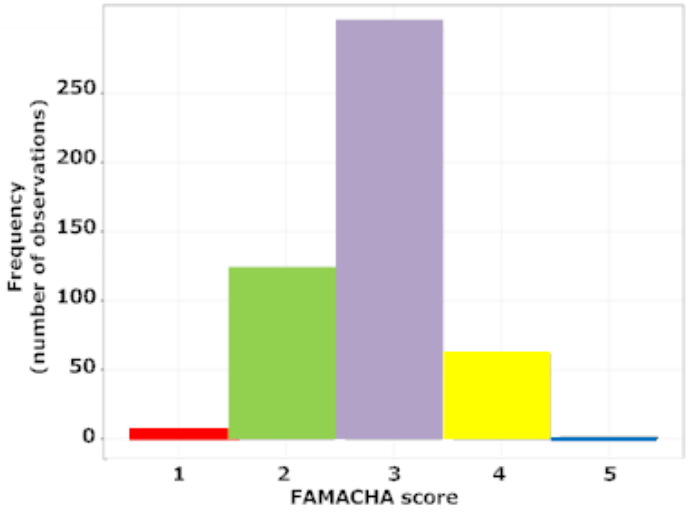
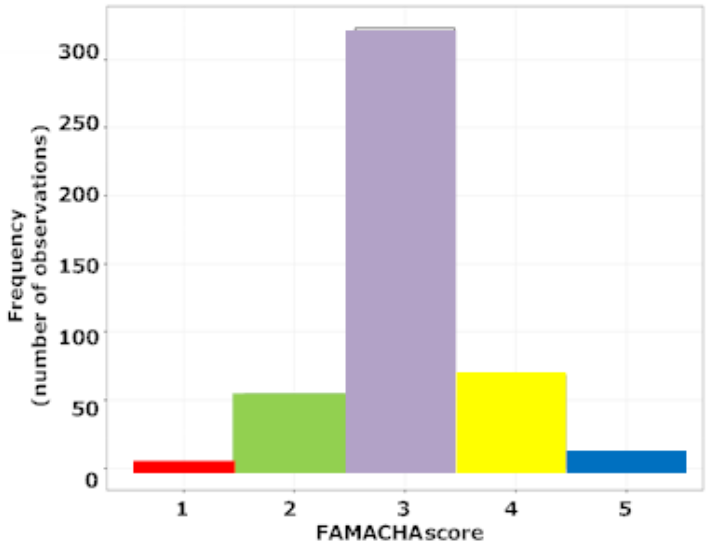
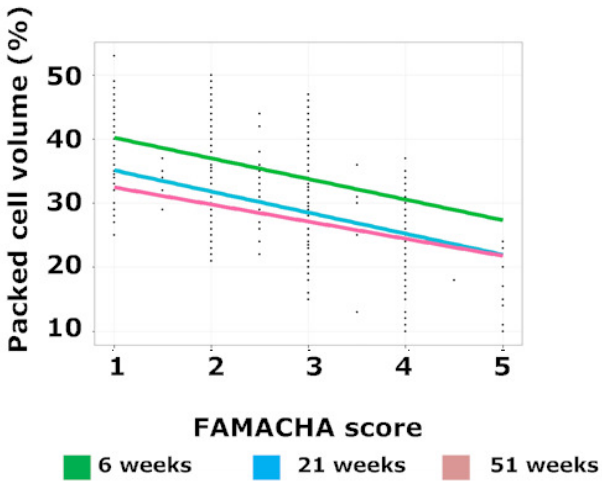


Figure 3.3c The frequency distribution of FAMACHA© scores for the 51-weeks age groups



The linear relationship between FAMACHA© score and PCV for each of the three age groups is given in Figure 3.4. The predicted PCV for each FAMACHA© score was consistently higher for the 6-week age group compared to the other two age groups. The predicted PCV (%) for calves with a FAMACHA© score of 2 for the 6, 21, and 51-week age groups respectively were 42.47%; 31.74% and 29.76%. The predicted PCV (%) for calves with a FAMACHA© score of 3 for the 6-, 21-, and 51-week age groups were 33.82%; 28.52% and 27.12%, respectively.

Figure 3.4 The linear relationship between FAMACHA© score and PCV (%) for the age groups 6 weeks, 21 weeks and 51 weeks



3.1.3 The performance of FAMACHA© using different chart cut-off points

Table 3.1 depicts how the sensitivity and specificity of the FAMACHA© test to detect anaemia (PCV<25%) changed with the use of different FAMACHA© cut-off values. The majority of cases were classified correctly, be that either as anaemic or not anaemic, when using a FAMACHA© score cut-off of 5.

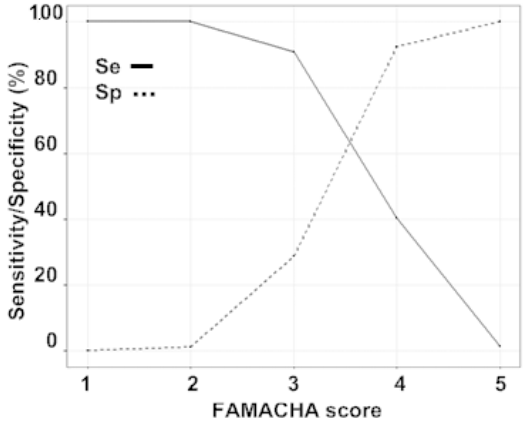
Table 3.1 Sensitivity and Specificity of FAMACHA© at different cut-off points (n=485)

Cut-off point	Se[1] (%)	Sp[2] (%)	Correctly classified (%)	LR+[3]	LR-[4]
1	100	0	15.9	1.00	Inf
2	100	1	16.7	1.01	0.0
3	90.9	28.7	38.6	1.27	0.312
4	40.3	92.4	84.1	5.30	0.646
5	13	100	84.3	Inf	0.99

[1] Se = sensitivity; [2] Sp = specificity; [3] LR+ = positive likelihood ratio; [4] LR- = negative likelihood ratio

The maximum test accuracy can be read off the two-graph ROC plot (Fig. 3.5) at the intercept of the Se and Sp curves (Reynecke *et al*. 2011). The Se-Sp intercept falls between a FAMACHA© score of 3 and 4. The LR+ at a FAMACHA© score of 4 (5.30) was considerably higher than at a score of 3 (1.27). It means that the odds of the animal being truly anaemic was over four times higher when a FAMACHA© cut-off of 4 was used

Figure 3.5 The two-graph receiver operating characteristic curve plot for the FAMACHA© test using a PCV<25% cut-off (n=485)



compared to a cut-off of 3. The Se at a FAMACHA© score of 4 (Se=40.3 %) was, however, considerably lower than at a FAMACHA© score of 3 (Se=90.9 %). Sensitivity <50% implies that an anaemic animal (PCV<25%) is more likely to be missed than diagnosed as positive.

3.1.4 The performance of FAMACHA© using different PCV cut-off points

In Table 3.2 the Se and Sp of the FAMACHA© test in detecting anaemia using a cut-off of PCV<25% is compared to Se and Sp of the test in detecting anaemia using a cut-off of PCV<21%. Using the FAMACHA© cut-off of 4 to detect anaemia with a cut-off of PCV<21% resulted in a higher Se but slightly lower Sp than when using a cut-off of PCV<25% anaemia. The PV+ and PV- for detecting PCV<21% were lower than in detecting PCV<25%, but the results were affected by the lower prevalence of cases with PCV<21%. The accuracy of the FAMACHA©, as measured from the two-graph ROC curve plot (Fig. 3.6), when used to detect PCV<21% was similar to the accuracy of the test when used to detect PCV<25%, as indicated by the intercept of the Se and Sp curves that fell between a score of 3 and 4.

Table 3.2 Comparison of the performance of FAMACHA© (cut-off = 4) using different PCV cut-off points (PCV<25% vs. PCV<21%)

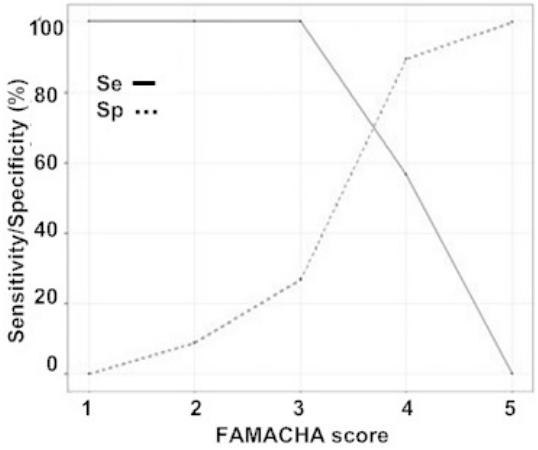
	PCV<25%		**PCV<21%**	
	Mean	**95%CI**	**Mean**	**95%CI**
Prevalence (%) of cases with PCV below cut-off points at 21 weeks age	15.88 ($n_{pcv<25}$=77/485)	12.61 to 19.14	4.74 ($n_{pcv<21}$=23/485)	2.84 to 6.64
Sensitivity (%)	40.50	33.13 to 44.87	56.52	52.11 to 60.93
Specificity (%)	92.4	90.04 to 94.76	89.39	86.65 to 92.13
PV+ (%)[1]	50	45.55 to 54.45	20.97	17.35 to 24.59
PV- (%)[2]	89.1	86.32 to 91.87	97.64	95.48 to 98.52

[1] PV+ = positive predictive value; [2] PV- = negative predictive value

3.1.5 Inter-rater performance of the FAMACHA® scoring system

The inter-rater performance of the FAMACHA® is illustrated in Table 3.3 and Figure 3.7. There was considerable variation between the performances of the different raters. Rater E showed the highest Se of 66.67%. Rater A had the highest Sp at 100%. Rater D correctly classified the most calves (88.24%). All raters had an AUC > 0.5 which implies that raters were able to discriminate between anaemic and non-anaemic animals using the

Figure 3.6 The two-graph receiver operating characteristic curve plot for a cut-off PCV<21% (n=485)



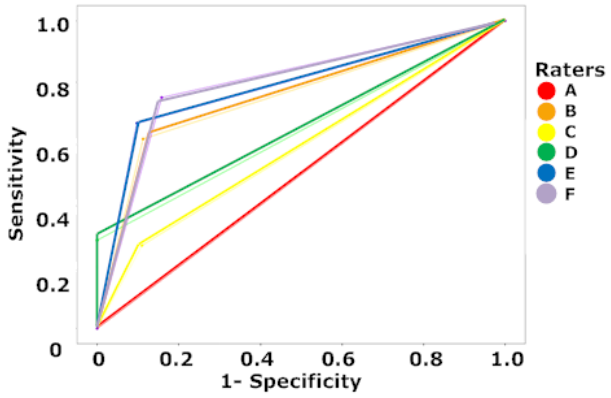
FAMACHA© test. The rater accuracy was low (AUC ≤ 7) for raters C and D, while the other raters were only moderately accurate (0.7 < AUC ≤ 0.9). Rater F was the most accurate with the highest AUC = 0.796.

Table 3.3 Inter-rater performance of the FAMACHA© scoring system

Rater	n	Se[1] (%)	Sp[2] (%)	Correctly classified (%)	PV+ [3] (%)	PV- [4] (%)	ROC area		
							AUC[5]	95%CI	
A	34	100	100	100	100	100	1	1	1
B	111	61.54	88.78	85.59	42.11	94.57	0.752	0.616	0.887
C	174	27.03	89.05	75.86	40	81.88	0.58	0.504	0.657
D	85	28.57	100	88.24	100	87.65	0.643	0.525	0.761
E	57	66.67	90.2	87.72	44.44	95.83	0.784	0.591	0.977
F	23	75	84.21	82.61	50	94.12	0.796	0.569	1.00

[1] Se = sensitivity; [2] Sp = specificity; [3] PV+ = positive predictive value; [4] PV- = negative predictive value; [5] AUC = area under curve

Figure 3.7 Receiver operating characteristic (ROC) curves of the various FAMACHA© score -raters



3.1.6 Comparison between FAMACHA© score-rater performances at different levels of experience

Rater A only made observations during the second year of the study and was thus excluded from this analysis. The accuracy of the raters B, E and F, as measured by the AUC of the ROC area, increased remarkably from the first half of the study to the second half, when they were more experienced. The accuracy of raters C and D did not change significantly and remained low (AUC < 0.7). Raters E and F improved their accuracy from moderate (0.7 < AUC ≤ 0.9) to highly accurate (AUC > 0.9). The Se of all the raters decreased from the first year to the second year, except for rater D who maintained a Se=100%. The Sp of all raters increased, except rater D who showed a decrease in Sp from 30% to 25% (Table 3.4).

3.2 Evaluation of the performance of the Sysmex automated analyzer

3.2.1 Comparison between the Sysmex and the Cell-Dyn® 3700

When comparing the mean differences of each parameter the Sysmex had higher readings (mean $\Delta<0$) than the Cell-Dyn® for lymphocyte counts, total WCC and MCHC; and lower readings (mean $\Delta>0$) than the Cell-Dyn® for other WCC counts,. RCC, HGB, HCT, MCV and Plt counts (Table 3.5). The limits of agreement for these parameters as measured by the Sysmex and the Cell-Dyn® are tabulated in Table 3.6. The intraclass correlation between these two analyzers is tabulated in Table 3.7.

Table 3.4 Comparison between FAMACHA© score-rater performances at different levels of experience

Rater	Year	n	Se[1] (%)	Sp[2] (%)	ROC[3] area		
					AUC[4]	95%CI	
B	1	74	95.31	50	0.727	0.567	0.884
	2	37	76.47	100	0.882	0.811	0.954
C	1	62	90.7	26.32	0.585	0.477	0.693
	2	112	88.3	27.78	0.58	0.472	0.689
D	1	52	100	30	0.65	0.508	0.792
	2	33	100	25	0.625	0.413	0.837
E	1	22	94.74	33.33	0.64	0.369	0.912
	2	35	87.5	100	0.938	0.88	0.995
F	1	11	87.50	66.67	0.771	0.481	1.00
	2	12	81.82	100	0.909	0.795	1.00

[1] Se = sensitivity; [2] Sp = specificity; [3]ROC = Receiver operating characteristic curve; [4]AUC = area under curve

Table 3.5 Within subject comparison of Cell-Dyn® and Sysmex

Parameter		Cell-Dyn®		Sysmex		Difference (Δ)*				
	n	Mean	SD	Mean	SD	Mean Δ	SD Δ	p-Value**	95% CI	
Lymph %	78	59.269	9.388	60.464	8.15	-1.195	3.167	0.0013	-1.909	-0.481
Other WBC %	78	40.731	9.388	39.536	8.15	1.195	3.167	0.0013	0.481	1.909
WCCx10^3/µL	77	11.6	3.56	11.523	3.29	0.077	0.606	0.266	-0.06	0.215
RCCx10^6/µL	78	8.446	0.793	8.432	0.77	0.014	0.142	0.391	-0.018	0.046
HGB g/dL	78	13.682	1.28	13.45	1.21	0.232	0.181	<0.001	0.191	0.273
HCT %	78	38.88	3.731	36.908	3.46	1.974	0.743	<0.001	1.807	2.142
MCV fL	78	46.118	3.11	43.88	3.21	2.238	0.704	<0.001	2.08	2.4
MCHC g/dL	78	35.245	0.732	36.463	0.9	-1.218	0.802	<0.001	-1.4	-1.04
Plt x 10^3/µL	78	484.11	164.107	432.35	157.38	51.76	49.572	<0.001	40.585	62.938

* Δ= CellDyn – Sysmex; **p-Value associated with Student's paired t-test

Table 3.6 Upper (UL) and lower limits (LL) of agreement between Cell-Dyn® and Sysmex

	Limits of agreement	
	LL=Mean(Δ) - 2SD*(Δ)	**UL=Mean(Δ) + 2SD(Δ)**
Lymph x 10^3/µL	-7.53	5.139
Other WBC x 10^3/µL	-5.139	7.53
WCC x 10^3/µL	-1.135	1.289
RCC x 10^6/µL	-0.27	0.298
HGB g/dL	-0.13	0.594
HCT %	0.488	3.46
MCV fL	0.83	3.646
MCHC g/dL	-2.822	0.386
Plt x 10^3/µL	-47.382	150.906

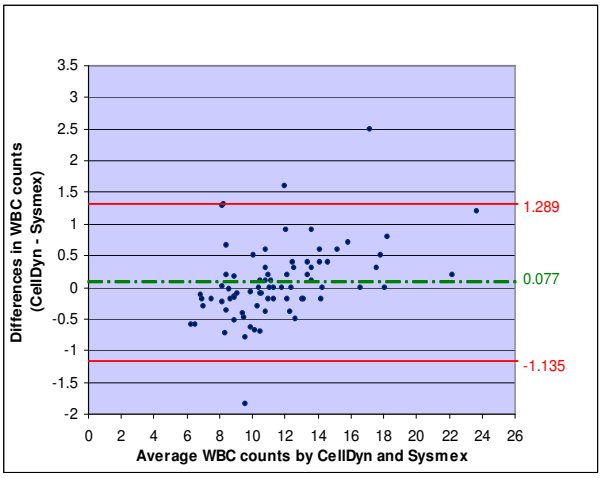
Table 3.7 Intraclasss correlation (ICC) between Cell-Dyn® and Sysmex

	ICC	**SE***	**95%CI**	
Lymph x 10^3/µL	0.927	0.016	0.896	0.958
Other WBC x 10^3/µL	0.927	0.016	0.896	0.958
WCC x 10^3/µL	0.984	0.003	0.977	0.991
RCC x 10^3/µL	0.984	0.004	0.976	0.99
HGB g/dL	0.972	0.006	0.96	0.985
HCT %	0.84	0.033	0.775	0.906
MCV fL	0.756	0.049	0.66	0.851
MCHC g/dL	0.00	0.11	0	0.22
Plt x 10^3/µL	0.904	0.02	0.863	0.945

*standard error of ICC

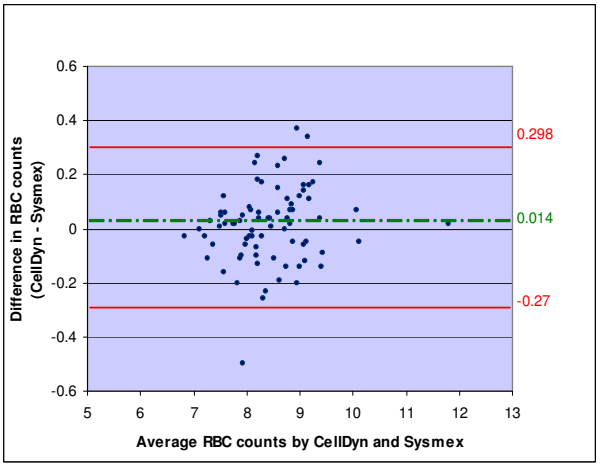
The mean Δ for both total WCC and RCC was not significant (P>0.1). The limits of agreement for WCC (-1.135; 1.289) x10^3/µL and RCC (-0.27; 0.298) x10^6/µL indicated that there was good agreement between the measurements using the Sysmex and Cell-Dyn® for these two parameters. The limits of agreement for WCC are illustrated in Figure 3.8 and for RCC in Figure 3.9.

Figure 3.8 Limits of agreement plots for white cell counts (x10^3/µL)



* mean Δ ; **UL & LL

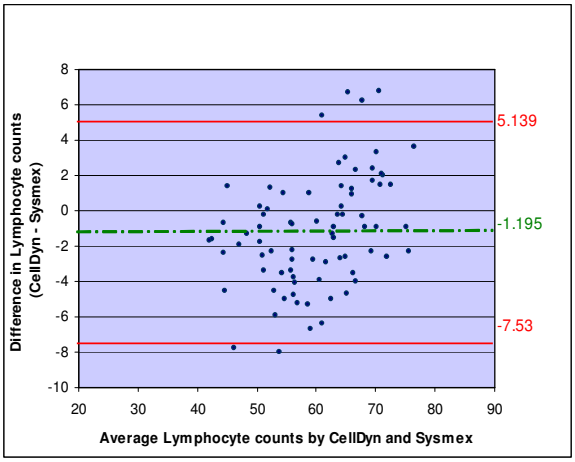
Figure 3.9 Limits of agreement plots for red cell counts (x10^6/µL)



* mean Δ ; **UL & LL

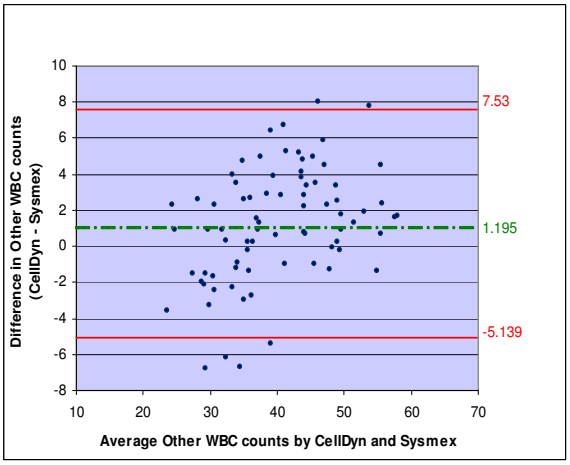
Differential WCC was reported by the Sysmex as either lymphocyte counts (% of total WCC) or other white blood cells (WBC) [WCC (100%) – lymphocyte (%)]. The limits of agreement for the two methods for lymphocyte counts (-7.53; 5.139) and for other WBC (-5.139; 7.53) indicated little agreement between the two methods. The Sysmex tended to give a higher reading than Cell-Dyn® for lymphocyte counts (between 1.909 and 0.481%) and a lower reading for other WBC (between 0.481 and 1.909%). The difference in means was significant (p=0.0013), but was of no clinical significance. The limits of agreement for lymphocyte counts are illustrated in Figure 3.10 and for other WBC in Figure 3.11.

Figure 3.10 Limits of agreement plots for lymphocyte relative counts (%)



* mean Δ; **UL & LL

Figure 3.11 Limits of agreement plots for other white cell relative counts (%)

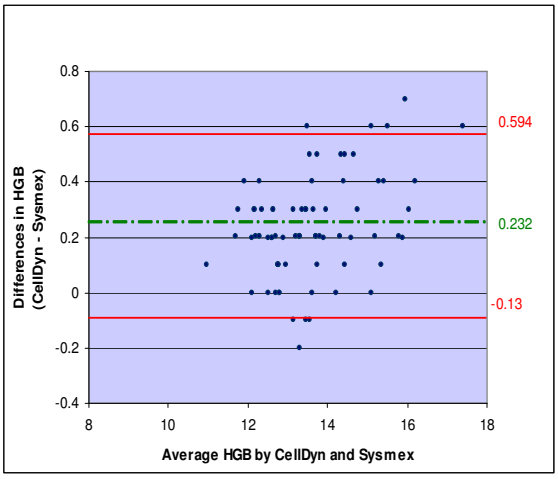


* mean Δ ; **UL & LL

There was good agreement between the two methods for HGB (limits of agreement = (-0.13; 0.594) g/dL) (Fig. 3.12). The difference in means between the two methods for HGB readings was significant ($p<0.001$), with the Sysmex consistently reading lower than the Cell-Dyn® by 0.191 and 0.273 g/dL. However, this was of no clinical significance.

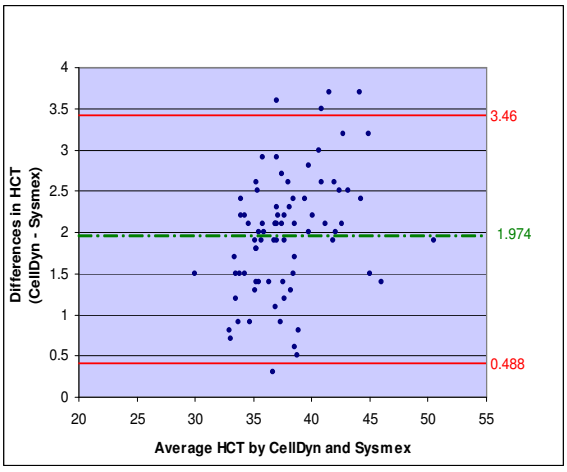
The Sysmex reading for HCT was lower than Cell-Dyn® (between 1.807 and 2.132%). The mean Δ was significant ($p=0$). The limits of agreement (0.488; 3.46) % were not clinically acceptable (Fig. 3.13). The ICC=0.84, indicating that some variation between the two sample sets was due to within-method variation as well as between-method variation.

Figure 3.12 Limits of agreement plots for haemoglobin concentration (g/dL**)**



* mean Δ ; **UL & LL

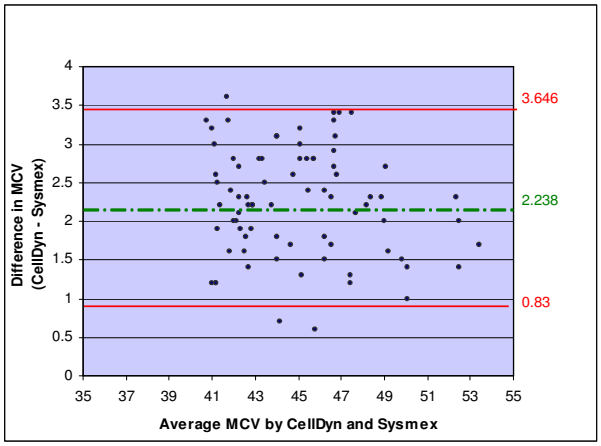
Figure 3.13 Limits of agreement plots for haematocrit (%)



* mean Δ ; **UL & LL

The mean Δ for MCV (2.238fL) was significant (p=0), with the Sysmex consistently reading lower than the Cell-Dyn® by between 2.08 and 2.4 fL. The limits of agreement (0.83; 3.646) fL indicated that there was no acceptable agreement between the two methods for this parameter (Fig. 3.14). Intraclass correlation (0.754) indicated that there was some variation between the two methods, as well as within the two sample sets. These results reflected the fact that MCV is a function of both HCT and RCC [MCV=HCTx10/RCC] and could thus have been affected by the readings for these two parameters.

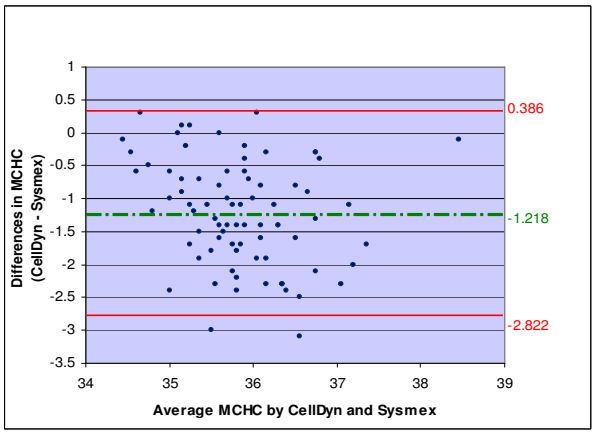
Figure 3.14 Limits of agreement plots for mean corpuscular volume (fL)



* mean Δ ; **UL & LL

Similarly, MCHC is a function of HGB and HCT [MCHC= (HGB x 100)/HCT] and can thus be affected by readings for both these parameters. The agreement was not good with limits of agreement of (-2.822; 0.386) (Fig. 3.15). The Sysmex reading was lower than the Cell-Dyn® reading by between 1.04 and 1.14 g/dL.

Figure 3.15 Limits of agreement plots for mean corpuscular haemoglobin concentrations (g/dL)

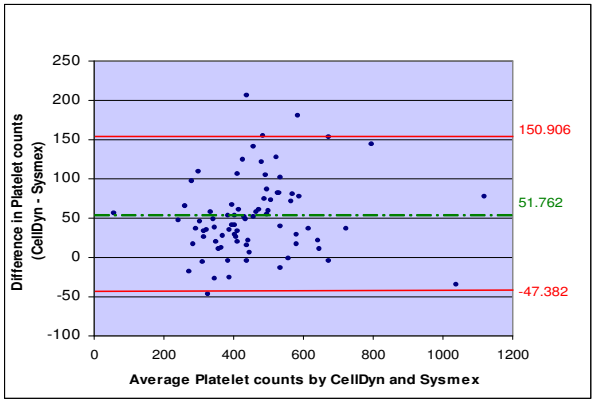


* mean Δ ; **UL & LL

The mean Δ in Plt counts between the two methods was significant ($p<0.001$), with the Sysmex reading lower than the Cell-Dyn® by between 40.59 and 62.9 x 10^3/µL. When evaluating the limits of agreement (-47.382; 150.906) x 10^3/µL, it indicated poor agreement between the two methods for this parameter (Fig. 3.16). The ICC also indicated that the variation was mainly due to inter-assay variation relative to intra-assay variation. However, the scale of the measurement should be considered here. Published reference values for Plt

counts (Jain 1993) give Plt values in units of 10^5/µL. If the Sysmex and Cell-Dyn® values are converted to units of 10^5/µL, the mean Δ =0.52 x 10^5/µL (95%CI = (0.41x10^5; 0.63x$10^{5)}$) and the limits of agreement of (-0.47 x 10^5; 1.5) x 10^5/µL, which indicates good agreement and is in accordance with published reference values for cattle (Jain 1993) and is clinically acceptable.

Figure 3.16 Limits of agreement plots for platelet counts (x10^3/µL)



* mean Δ ; **UL & LL

3.2.2 Comparison between the Sysmex HCT and manual PCV

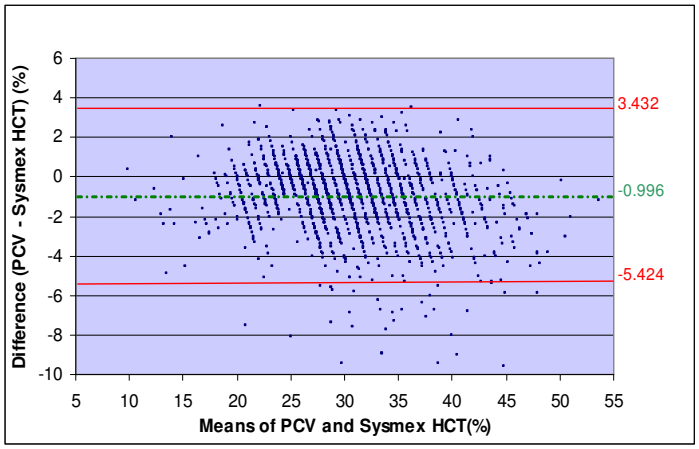
The Sysmex HCT generally tested higher (mean Δ = -0.996) than the manual PCV (Table 3.8). The agreement between PCV and HCT was not good, with limits of agreement of (-5.424; 3.432) (Fig. 3.17). The manufacturer's manual (Ginder 2007) recommends that a correction factor (CF) should be calculated to correct for a difference between PCV and HCT by calculating the ratio of PCV/HCT for each pair of values. The mean ratio (excluding values >mean+2SD and <mean-2SD) equalled the correction factor, which, for this data set, was measured as 0.987. There was improved agreement between PCV and the corrected HCT (Fig. 3.18). The mean Δ was insignificant (p=0.424).

Table 3.8 Within subject comparison of PCV and Sysmex HCT and PCV and the corrected HCT

	Mean	**Difference (Δ)***				
	(SD)	**Mean Δ**	**SD Δ**	**p-Value****	**95% CI**	
PCV (%)	30.19 (6.14)					
Sysmex HCT (%)	31.18 (6.73)	-0.996	2.214	<0.001	-1.436	-0.556
Corrected HCT (%)	30.34 (6.44)	-0.18	1.799	0.424	-0.601	0.255

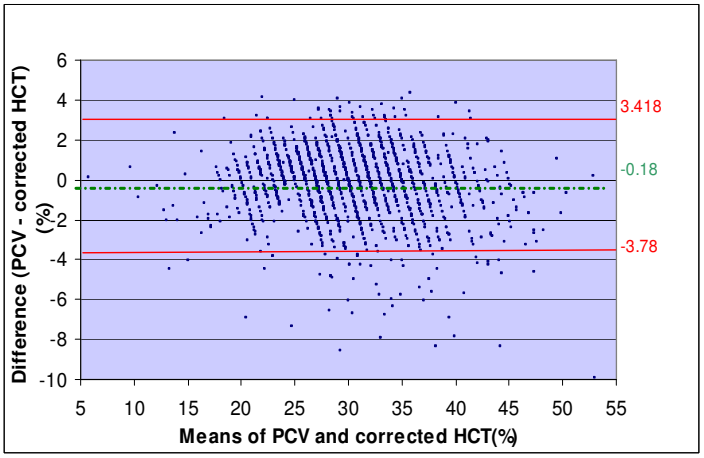
* Δ= PCV – HCT; **p-Value associated with Student's paired t-test

Figure 3.17 Limits of agreement plot for packed cell volume and Sysmex haematocrit



* mean Δ ; **UL & LL

Figure 3.18 Limits of agreement plot for packed cell volume and the corrected haematocrit



* mean Δ ; **UL & LL

4. DISCUSSION

4.1 Evaluation of the field performance of the FAMACHA©

The value of the FAMACHA® ocular mucosa chart lies in its simplicity and low cost, which both contribute to its value as a field screening-test that can be used by laymen. Two drawbacks to this ocular mucosa chart are its relatively low sensitivity and variation in the performance between raters. In order for a test to be considered a good screening test, it has to have a high sensitivity. The sensitivity can be increased by narrowing the inclusion criteria for anaemia, for example from PCV<25% to PCV<21%, but at this PCV the animal is already likely to be in critical need of intervention. The sensitivity can also be increased by

changing the cut-off of the FAMACHA® from 4 to 3. From the two-graph ROC plot the point of maximum accuracy of the test falls at a point between a score of 3 and 4, for both PCV cut-off points (<25% and <21%). A FAMACHA© score of 3 appears to be the optimal choice to maximize the accuracy as a FAMACHA© cut-off of 4 would reduce the Se of the test considerably and therefore a high number of positive cases would be misdiagnosed as negative. The accuracy of the scoring systems for most raters improved as they became more experienced. This came at a cost of a lower sensitivity in most raters. The age of the animal needs to be taken into account, however, as it was shown in this study that there was a difference in the PCV range corresponding to each FAMACHA® score between the different age groups. The younger age group had a higher mean PCV for each FAMACHA© score.

4.2 Evaluation of the performance of the Sysmex automated analyzer

Compared to the Cell-Dyn® 3700 automated analyzer, the Sysmex showed clinically acceptable agreement with regards to white blood cell parameters. This is in agreement with a similar study that compared the performance of the two analyzers, specifically using the optical channel method of the Cell-Dyn 3500 to measure bovine white cell parameters in bovines (Riond, Weissenbacher, Hofmann-Lehmann & Lutz (2011).

The two analyzers did not show good agreement on platelet counts in this study, but the difference was still within published reference ranges and was considered clinically acceptable. Riond *et al*. (2011) found a negative bias in PLT counts in bovine samples, but considered it to be not of any clinical importance as well. There was also good agreement between the two analyzers on RCC and HGB in this study. This too, is in agreement to what was found by Riond *et al*. (2011) for these two parameters in bovines.

There was poor agreement between the Sysmex and the Cell-Dyn® on HCT measurements. The difference was clinically significant. This poor agreement also led to poor agreement between the two analyzers on MCV and MCHC since both these parameters are not measured directly by the Sysmex but calculated from red blood cell indices (RCC, HCT and HGB) (Ginder 2007; Riond *et al*. 2011). There was also poor agreement between the Sysmex HCT and manual PCV, which was improved when a correction factor was taken into consideration. This difference is likely due a difference in methodology, as PCV was measured directly using the microhaematocrit method, whereas the HCT was calculated using the RCC pulse-height detection method by the Sysmex (Ginder 2007; Riond *et al*. 2011). The correction factor calculated could not be extrapolated for use in the comparison

to the Cell-Dyn®, however, since a different group of cattle were sampled. It would probably have been worthwhile to measure the manual PCV for this purpose and assess whether the corrected HCT would have improved the agreement between the Sysmex and Cell-Dyn® for MCV and MCHC as well.

5. CONCLUSIONS

The FAMACHA© test was designed for use in sheep. The reference ranges of PCV differ between species and this necessitates the calibration of the test for the species, such as cattle, before it is applied to that species. Moors & Gauly (2009) found that mucosa colour of different sheep breeds differ, and this is probably true for cattle breeds as well. They suggested adapting the FAMACHA© colour scales for different breeds to increase its validity. Such an adaptation for various species, breeds and age-groups within breeds might also be appropriate.

One has to weigh the benefits and costs of missing truly diseased animals by using a test with lower Se on the one hand and on the other hand the unnecessary treatment of false positive cases by using a test of high Se but low Sp. The unnecessary treatment of false positive cases increases the cost of treatment and drugs and can also contribute to the development of drug resistance. In the rural setting of Western Kenya, individual animals will most likely only be inspected for specific clinical signs, such as pale mucosa, once they are already suffering from more general signs such as ill-thrift or loss of condition. In such cases, criteria should be selected to optimize the sensitivity of the FAMACHA© test, such as using a cut-off of 3, in order to screen whether the animal actually requires treatment.

Only the FAMACHA© tests' validity in diagnosing cases of anaemia was investigated in this study and not its value in predicting specific causes of anaemia, such as helminthosis or trypanosomosis. Mixed infections are common in the tropics and many infections present with similar clinical signs. Focusing the FAMACHA© test on specific pathogens in this setting might lead the investigator to under-diagnose super-infections with other pathogens. Identifying individual animals with anaemia is only the initial step in managing the diseased animal. Treatment of such cases should not commence without further diagnostics into the specific underlying causes.

Overall the FAMACHA© was acceptable as a field test in detecting anaemia in East African short-horn Zebu calves (Fig. 3.19). Proper training of raters, being the farmer, veterinarian or

an animal health officer, is essential in optimizing the sensitivity and accuracy of the FAMACHA© chart in the field. If anything, the implementation of this scoring system forces the farmer to assess the health status of each animal individually from close-by and therefore promote earlier recognition of ill-health and initiation of intervention.

Figure 3.19 FAMACHA© scoring in an East African short-horn Zebu calf