

ارزیابی روش شیمیائی خشک (سیستم Reflotron^R) جهت آنالیز نمونه‌های خون در گاوان شیری

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خلاصه:

نمونه‌های خون از ۸۵ راس گاو هلشتاین فریزین تهیه شده جهت آسپاریت آمینوترانسفراز (AST). آلانین آمینوترانسفراز (ALT)، گاما گلو تامیل ترانسفراز (GGT)، گلوکز (GLU)، ازت اوره خون (BUN)، کلسترول تام (T-CHOL)، تری گلیسرید (TG)، اسیداوریک (UA) و بیلروبین تام (T-BIL) با استفاده از روش شیمیائی خشک یا سیستم Reflotron مورد آنالیز قرار گرفتند. سرمهای بدست آمده از همین نمونه‌های خون جهت تستهای مشابه با استفاده از یک روش شیمیائی مرطوب یا تجزیه گر سریع خون (RaBA- Σ^R) مورد آنالیز قرار گرفتند. Reflotron قادر نبود شاخصهای UA، TG، T-CHOL-T-BIL را بطور دقیق مشخص نماید چراکه اکثر آنها در پائین تر از حد قابل تشخیص برای دستگاه قرار می‌گرفتند. متوسط AST از سیستم Reflotern ۱/۶ مرتبه بیشتر از رقم حاصل از روش RaBA بود. متوسط GLV از سیستم RaBA ۱/۱ مرتبه بیشتر از عدد حاصل از روش Reflotron بود. ضرایب همبستگی بالا برای AST (۰/۹۴۷۷)، GLV (۰/۹۶۱۵)، ALT (۰/۹۱۲۱)، BUN (۰/۹۱۶۰)، GGT (۰/۸۷۰۲) از دو سیستم Reflotron و RaBA بدست آمدند. نتایج نشان می‌دهند که سیستم Reflotron بخوبی قابل مقایسه با یک روش مرطوب مثل RaBA با دقت بالا جهت آنالیز AST، ALT، GGT، GLV، نمونه‌های خون در گاوان شیری می‌باشد و می‌تواند جهت تجزیه این پارامترهای خونی مورد استفاده قرار گیرد.

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References :

- 1) Baumgartner W, Schlerka G, Jahn J: First report on the use of the Reflotron system (dry reagent tests) for laboratory diagnosis in cattle. *Wiener Tierarztliche Monatsschrift* 73:409-412, 1986.
- 2) Belford CS, Lumsden JH: Assessment of a reflectance photometer in a veterinary laboratory. *Can Vet J* 25:243-246, 1984.
- 3) Bent E, Hambitzer R: New uses for a dry-reagent analytical test system. I. Determination of enzymes, metabolites and minerals in the blood of horses. *Berliner Munchener Tierarztliche Wochenschrift* 100:418-422, 1987.
- 4) Beyer C, Carstensen C, Grunert E: Measurement of asparatate aminotransferase activity in bovine blood by the " Reflotron " system. *Praktische Tierarzt* 70:16-20, 1989.
- 5) Chalifoux A, Tremblay A: Evaluation of a dry reagent chemistry system (Reflotron) in the dog. *Medecin Veterinaire du Quebec* 17:87-90, 1987.
- 6) Dollin BE: Observations on the use of the " Seralyzer " solidphase clinical chemistry system in a veterinary hospital laboratory. *J Small Anim Pract* 27:279-288, 1986.
- 7) Eckersall PD, Gribben S, Marshall C: Assessment of a dry chemistry analyser for use with canine blood samples. *Vet Record* 124:427-428, 1989.
- 8) Gruber W, Werner W, Rittersdorf W, Kamlah H: Real time analysis with the Reflotron^R/Refloquant^R system. The combination of dry chemistry with refiometry. *Lab Clin Pract* 2:77-83, 1984.
- 9) Hirschberger J, Scholz U: Modern dry reagent chemistry in veterinary medicine, as represented by two systems (Reflotron, Ektachem). *Tierarztl Umschau* 44:431-434, 1989.
- 10) Sakamoto Y, Miyazawa Y, Ando H: Evaluation of multilayered dry chemistry system for canine blood chemistry test. *J Jpn Vet Med Assoc* 44:1121-1128, 1991.

Table 3. Correlation between Reflotron^R-System and RaBA- Σ^R .

Test	n	Correlation coefficient	Regression equation ^a
AST	85	0.9477	y= 13.1695 + 1.4668x
ALT	85	0.9121	y= -3.7285 + 1.2083x
GGT	85	0.8702	y= 2.4139 + 0.9898x
GLU	85	0.9615	y= 9.9696 + 0.7919x
BUN	79	0.9160	y= 2.2597 + 0.8523x

^ay is the result from Reflotron^R-System and x is the result from RaBA- Σ^R .

Table 1. Range of different tests for Reflotron^R-System

Test ^a	Unit	Range
GLU	mg/dl	10.0 - 660.0
HB	g/dl	5.0 - 20.0
T-CHO	mg/dl	100.0 - 500.0
TG	mg/dl	70.0 - 600.0
GGT	U/l	5.0 - 3,500.0
BUN	mg/dl	9.0 - 140.0
AST(GOT)	U/l	5.0 - 1,500.0
ALT(GPT)	U/l	5.0 - 2,000.0
UA	mg/dl	2.0 - 20.0

^aGLU: glucose, HB: hemoglobin,
TG : triglyceride,
BUN : blood urea nitrogen,
ALT : alanine aminotransferase,

T-CHO: total cholesterol,
GGT: gamma-glutamyl transferase,
AST : aspartate aminotrans-ferase,
UA : uric acid.

Table 2. Comparison of the results between ReflotronR-System and RaBA- Σ^R

Test	unit	nc	Range		Mean		S.D. ^a		C.V. ^b (%)	
			Ref. ^d	RaBA ^e	Ref.	RaBA	Ref.	RaBA	Ref	RaBA
AST	U/l	85	36-945	41-588	209.1	133.5	191.4	123.6	91.5	92.5
ALT	U/l	85	7-87	9-77	38.5	35.0	17.4	13.1	45.2	37.4
GGT	U/l	85	12-52	12-46	26.6	24.7	7.7	7.1	28.9	28.7
GLU	mg/dl	85	57-190	52-244	85.4	93.4	27.3	33.1	32.0	35.4
BUN	mg/dl	79	9-30	7-32	12.9	12.5	3.5	3.8	27.1	30.4

^aS.D. : standard deviation.

^bC.V. : coefficient of variation.

^cn : number of animals.

^dRef. : Reflotron -System.

^eRaBA : RaBA- Σ^R .

Baumgartner et al (1) can be attributed to the wider range of observations in this study, since blood samples were taken not only from apparently healthy cows but also from diseased animals.

The results from Reflotron^R and RaBA- Σ^R yielded high correlation coefficients (Table 3). GLU had the highest correlation (0.9615) followed by AST (0.9477). These two tests also had the widest ranges. The correlation for ALT and BUN were 0.9121 and 0.9160, respectively. GGT gave the lowest correlation at 0.8702.

Baumgartner et al (1), in their study on dairy cows, obtained a correlation of 0.912 for GLU and relatively high correlation of 0.945 for GGT. Hirschberger and Scholz (9), on the other hand, in their study on dogs, obtained a correlation of 0.8558 for ALT, 0.9898 for AST, and 0.972 for GLU. For cats, the correlations were 0.9694 for ALT, 0.9442 for AST and 0.922 for GLU. Also in dogs, Echersall et al (7) obtained a relatively lower correlation for AST of 0.87 and a higher correlation for ALT of 0.98; correlations for GGT and GLU were 0.83

and 0.92, respectively. Also in dogs, Chalifoux and Tremblay (5) also observed good reliability of blood values of blood glucose, BUN, cholesterol and hemoglobin obtained by Reflotron compared with an automatic analyser.

The above results show that Reflotron^R-System compares well with that of wet-reagent method, RaBA- Σ^R , in terms of high precision for analysis of AST, ALT, GGT, and GLU of blood samples in dairy cows and can be used for the analysis of these blood constituents. The relatively higher values for AST and the lower values for GLU compared with the wet-reagent method used and it should always be borne in mind when using Reflotron^R, reference values for the Reflotron^R-System should always be used. Reflotron^R can also be used for analysis of BUN but only to a limited degree, as for screening test, bearing in mind that low values are not accurately measured with this method. Finally the high lower limits for T-CHO, TG, T-BIL and UA restricts the use of Reflotron^R-System for analysis of these blood constituents in dairy cattle.

suffering from various disorders and brought to a slaughterhouse in Obihiro City, Tokachi District, Hokkaido Prefecture, Japan, from June to December, 1991. Blood was collected using both heparinized and non-heparinized vacuum tubes. Heparinized blood was immediately analyzed for AST, ALT, GGT, GLU, BUN, T-CHO, TG, UA and T-BIL using a dry-reagent chemistry, Reflotron^R-System (Boehringer Mannheim Yamanouchi Co. Ltd., Tokyo, Japan). Non-heparinized blood was centrifuged. serum was extracted and analyzed for the same blood constituents using a wet-reagent chemistry, RaBA- Σ^R (Chugai Pharmaceutical Co. Ltd., Tokyo, Japan). The results from Reflotron^R and RaBA- Σ^R were compared, the means were analyzed using Student's T test and linear correlations were determined.

Results and discussion :

Table 1 lists the test range of different analytes for Reflotron^R as supplied by the manufacturer. Values for TG and UA fell below the lower limit for each test. Thus, Reflotron^R was not able to accurately determine the values for TG and UA. In the same manner, for T-CHO, 61 out of 85 cows had <100 mg/dl, for T-BIL, 68 out of 85 cows had <0.05 mg/dl and for BUN, 6 out 85 cows had <9.0 mg/dl. Since insufficient data for TG, UA, T-CHO and T-BIL were obtained on the Reflotron^R, no comparison for these blood constituents were made with the results obtained from RaBA- Σ^R .

Table 2 shows the results of analysis for AST, ALT, GGT, GLU and BUN from Reflotron^R and RaBA- Σ^R . The mean for GLU as measured by Reflotron^R was higher, the mean for GGT was similar, the standard deviation for GLU was higher while that for GGT was lower than corresponding figures in a previous study (1).

Comparing the results from Reflotron^R with that from RaBA- Σ^R , for AST, the values from Reflotron^R had a wider range than that obtained from RaBA- Σ^R . For GLU, the values from RaBA- Σ^R had a wider range than that obtained from Reflotron^R. The mean for AST from Reflotron^R (209.1 U/l) was 1.6 times higher than that obtained from RaBA- Σ^R (133.5 U/l), significantly different at $p < 0.01$. This agrees with the finding of Beyer et al. (4) who compared results from Reflotron^R with another wet-reagent chemistry (Gilford 203 S^R) using blood samples from 112 Black Pied cows. The mean for GLU from RaBA- Σ^R (93.4 mg/dl), on the other hand, was about 1.1 times higher than that obtained from Reflotron^R (85.4 mg/dl), but not significantly different at $p < 0.05$. For ALT, GGT and BUN, the figures for range and mean were similar and were not significantly different at $p < 0.05$.

Copmparison of the coefficients of variation of the results from Reflotron^R and RaBA- Σ^R showed similar figures (Table 2). The relatively high coefficients of variation as compared with those obtained by

Introduction :

Dry-reagent chemistry is a new development in clinical biochemistry which enables rapid analysis of blood by allowing direct application of a sample into a strip or slide containing dried reagents. In contrast with wet-reagent methods, dry-reagent chemistry can analyze whole blood as well as plasma or serum. In addition, the advantages of dry-reagent chemistry include simple handling, no sample dilution, results obtainable in 2-3 min, excellent correlation with reference methods, quality control and economic efficiency (1). The most common dry-reagent chemistry systems currently in use include: Ektachem^R-System (3,9), Seralyzer^R-System (2,6), Konica Dry Labo^R-System (10) and Reflotron^R-System (1,4,7). However, since these were developed originally for use in human medicine, precision studies comparing them with standard methods are necessary before they can be used for veterinary medicine.

The Reflotron^R-System consists of three components: photometer, test reagents and a special pipette for collecting and dosing the sample. By means of reagent carriers, the concept offers the user not only long term stability of the tests but also a shortening of the pre- and post-analytical steps such as:

a) use of integrated glass fiber separation instead of plasma separation by centrifugation; b) combination of different, pre-dosed amounts of ingredients in dry

chemistry layers instead of mixing reagents; c) calibration of instrument individually and automatically, carried out by a magnetic code on the back of each reagent carrier; and d) calculation of kinetic procedures processed by an in-built microprocessor system (8).

The first report on the use of Reflotron^R in dairy cows was made by Baumgartner et al (1) who analyzed urea, total cholesterol (T-CHO), glucose (GLU) and gamma-glutamyl transferase (GGT) of blood samples from 115 cows. Beyer et al (4) also reported the use of Reflotron^R for determination of aspartate aminotransferase (AST or GOT) in Black Pied cows. Eckersall et al (7) assessed the use of Reflotron^R to investigate alanine aminotransferase (ALT or GPT), AST, T-CHO, GGT, GLU, hemoglobin (HB) and urea of canine blood. Hirschberger and Scholz (9) also used Reflotron^R to analyze GLU, GGT, AST and ALT in dogs and cats. The present study was conducted to investigate the usefulness of a dry-reagent chemistry, Reflotron^R-System, in the analysis of AST, ALT, GGT, GLU, blood urea nitrogen (BUN), triglyceride (TG), T-CHO, uric acid (UA) and total bilirubin (T-BIL) in apparently healthy and diseased dairy cows.

Materials and methods :

Blood samples were taken from 40 apparently healthy dairy cows, aged 3 to 11 years, at the Obihiro University Dairy Farm and 45 dairy cows, aged 2 to 11 years,

Evaluation of dry - reagent chemistry (Reflotron^R - system) for analysis of blood samples in dairy cows

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Summary:

Blood samples were taken from 85 Holstein - Friesian cows in Tokachi District, Hokkaido Prefecture, Japan and analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma - glutamyl transferase (GGT), glucose (GLU), blood urea nitrogen (BUN), total - cholesterol (T-CHOL), triglyceride (TG), uric acid (UA) and total bilirubin (T-BIL) using a dry reagent chemistry, Reflotron^R-System. Sera from the same blood samples were extracted and analyzed for the same tests using a wet - reagent method, Rapid Blood Analyzer (RaBA- Σ^R). Values obtained from Reflotron^R-System and RaBA- Σ^R were then compared and analyzed.

Reflotron^R was not able to accurately determine the values for TG, UA, T-BIL, and T-CHOL since most of the values fell below the lower limit for each test. The mean for AST from Reflotron^R was 1.6 times higher than that obtained from RaBA- Σ^R . The mean for GLU from RaBA- Σ^R was 1.1 times higher than that obtained from Reflotron^R. High correlation coefficients for AST (0.9477), GLU (0.9615), ALT (0.9121), BUN (0.9160) and GGT (0.8702) were obtained from Reflotron^R and RaBA- Σ^R . The results show that Reflotron^R-System compares well with that of a wet-reagent method, RaBA- Σ^R , in terms of high precision for analysis of AST, ALT, GGT and GLU of blood samples in dairy cows and can be used for the analysis of these blood constituents. The high lower limits for T-CHO, TG, T-BIL and UA, on the other hand, restricts the use of Reflotron^R-System for analysis of these blood constituents in dairy cattle.

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