Development of the Quantitative Immunoassay Reagent for Measuring BA (Bile Acids) Levels in Dog and Cat "FUJI DRI-CHEM IMMUNO AU Cartridge v-BA"

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Abstract

We have successfully developed and commercialized the "FUJI DRI-CHEM IMMUNO AU Cartridge v-BA", which is a quantitative immunoassay system for measuring total bile acid levels in dog and cat serum and plasma. This reaction system allows the simultaneous measurement of three bile acids and has been successfully used to diagnose hepatobiliary disease with high accuracy in these animals.

1. Introduction

Recent development of veterinary medicine has succeeded in prolonging the average life span of pets. Meanwhile, there are diseases that tend to easily appear due to aging of pets. Hepatic and biliary tract diseases for dogs, for example, have the same tendency as liver diseases for human beings: they significantly increase after the age of 4 to 5, equal to the middle of 30s in human. Liver and gallbladder diseases for dogs appear from various causes such as congenial malformation or predisposition, viral or bacterial infection, intake of harmful substances, unbalanced meals, etc. Human liver is called as "the silent organ", which is true to dog's liver: the abnormality in physical conditions is difficult to recognize only by external appearance. Thus it is important for pets to undergo medical checkups, including blood, X-ray and echo inspections[1].

A blood inspection for a pet which is suspected to have a liver disease checks four hepatic enzymes as a primary panel: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and γ-glutamyltranspeptidase (GGT). However, blood inspections also inspect the secondary panel (liver function inspection) to evaluate liver functions to a certain level, as these enzymes do not reflect the functions. Secondary panel items include bile acids (BA), bilirubin, ammonia, albumin, cholesterol, urea nitrogen. BA, among them, is most superior in the sensitivity and peculiarity in checking liver functions for dogs and cats[2].

Generally there are two types of immunoassays to measure antigens such as hormones, utilizing immunological reactions through the use of antibodies: a sandwich method and a competitive method. The former is used for antibodies with the molecular weight at several ten thousands Da (like protein) and sandwich them with two antibodies, while the latter is used for antigens with the molecular weight at less than 1,000 (low molecular antigens like BA) too small to be sandwiched, and have antigen label and antigen in the specimen compete each other in immunological reactions.

Our company has developed the FUJI DRI-CHEM IMMUNO AU10V, an immunological analyzer for animals that can use both methods, and the FUJI DRI-CHEM IMMUNO AU Cartridge v-T4, vc-TSH, v-COR, dedicated immunoassay reagent and report on them[3][4]. This system adopts a surface plasmon-enhanced fluorescence (SPF) method as a principle to detect fluorescent particles to be used as markers. The conventional epifluorescence microscopy method whose laser beam illuminates uncombined fluorescent particles by immunological reactions, requires cleaning to eliminate unreacted fluorescent particles. The SPF method adjusts the incident angle of laser beams from the bottom surface, and irradiate them on a thin metal surface having combined fluorescent particles to generate proximity-field light through surface plasmon resonance (SPR), as a result of which only combined fluorescent particles on thin metal surface illuminates (Fig. 1). Elimination of cleaning has shortened the measurement time to about 10 minutes, and the system become more compact

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as it requires no cleaning agent and wastewater treatment system. It is also characterized by its easy operation: all you have to do is to set a specimen, cartridge, and consumables on the system and to press the start button (Fig. 2).

This time we have developed a new reagent, the FUJI DRI-CHEM IMMUNO AU Cartridge v-BA (FDC v-BA), using the competitive method to enable fast and simplified measurement for BA. In this paper, we will describe the technical overview and clinical performance of the FDC v-BA.

2. Development of FDC v-BA using the competitive method

2.1 Meaning of blood BA in blood measurement

BA are steroid derivatives generated from cholesterol in the liver, and "bile acid(s)" is a collective term for compound having cholanic acid structure. Therefore, BA are measured for the total volume as BA concentration. Its biological role

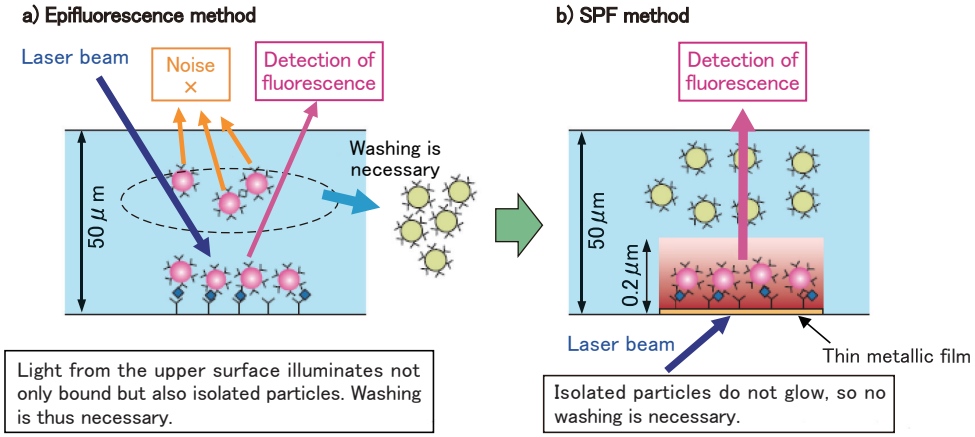

Fig. 1 Schematic representation of a) the epifluorescence method and b) the SPF method

Fig. 2 Quantitative immunoassay system for measuring BA levels in dog and cat serum and plasma

is related to digestion and absorption of fat, as well as absorption of liposoluble vitamins.

BA generated from the liver are concentrated in the gallbladder as bile, and sent to the duodenum. Then it is efficiently absorbed by the portal vein through the intestinal tract, absorbed in and re-excreted from the liver. In this way, BA circulate between the intestine and liver in an extremely closed manner (Fig. 3). Due to this, in-blood BA concentration is normally low[5]. If, on the other hand, there is any disorder in excretion into the bile, in the portal vein circulation path back to liver, and absorption in liver cells, BA inflow into the general circulation system, causing a high in-blood BA concentration[6].

One of disorders in the portal vein circulation path is portosystemic shunt (PSS). With PSS, portal vein blood inflows into the entire systemic circulation due to shunt blood vessels from portal veins to the entire body. At this point, neurotoxic substances absorbed from the intestinal tract directly flow into the peripheral and cerebral circulation systems without detoxified in the liver, posing a problem as it may cause impaired consciousness. Moreover, BA re-absorbed from the intestinal tract also directly flows in the general circulation system, causing in-blood BA at a high level[5]. Judging from the above, in-blood BA measurement is effective for diagnosing hepatic and biliary tract diseases.

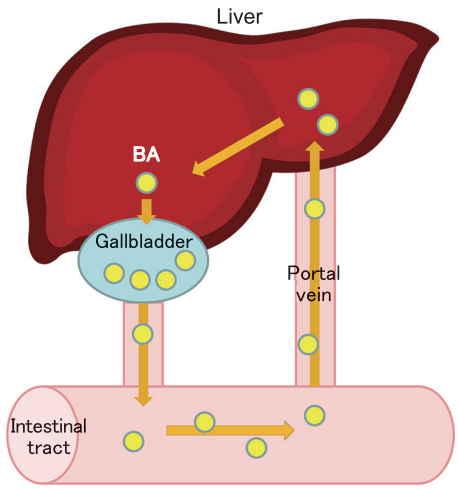

Fig. 3 Enterohepatic circulation of bile acids

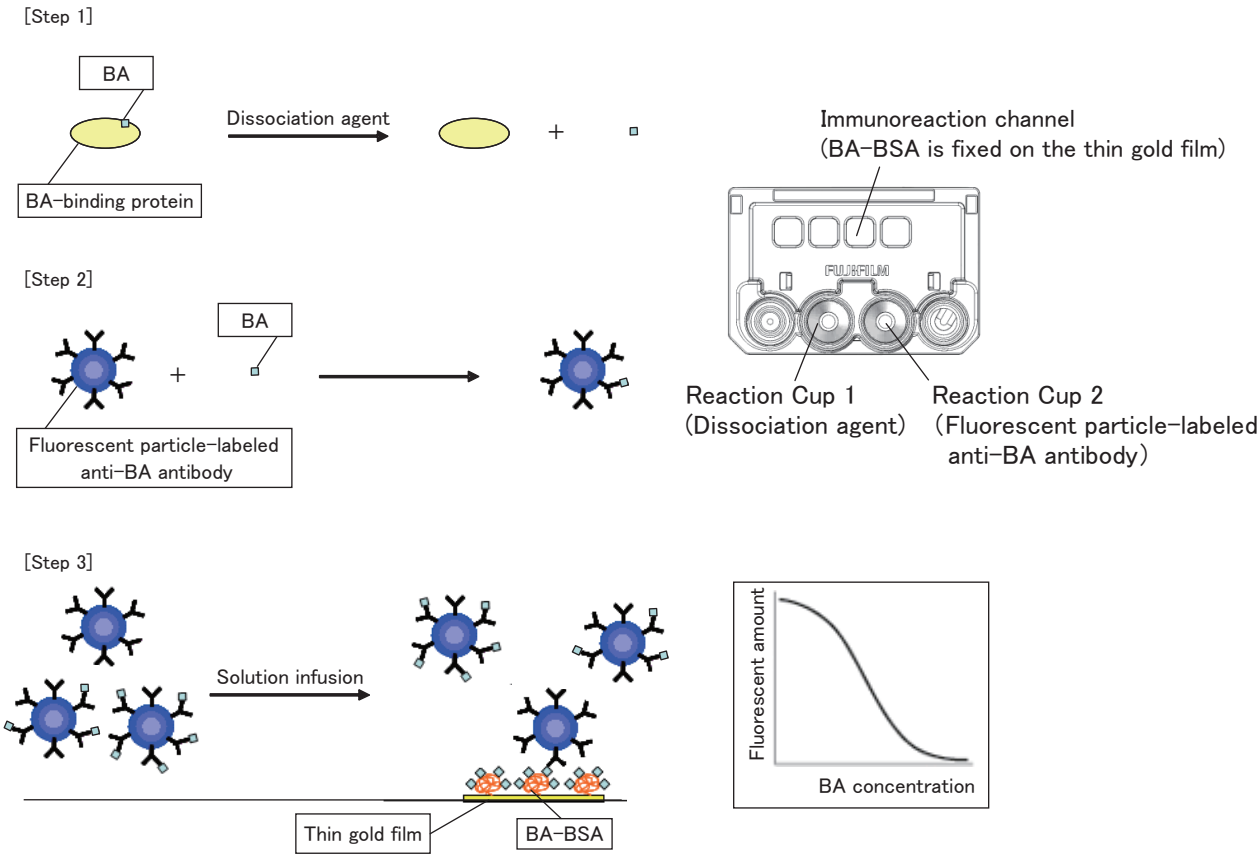

Fig. 4 Principles underlying the measurement of BA levels in dog and cat serum and plasma using FDC v-BA

2.2 Measurement principle for FDC v-BA

The FDC v-BA measurement using the competitive method is conducted as follows (Fig. 4).

(1) Specimens are dispersed in the Reaction Cup 1. Then BA-binding protein and dissociating agent in specimens react to each other while being dissolved to generate free BA (Step 1).

(2) The reaction solution in the Reaction Cup 1 is dispensed into the Reaction Cup 2, fluorescent particles marked with BA monoclonal antibody (fluorescent-labeled anti-BA antibody) react with BA in specimens (Step 2). At this point, the volume of BA combined with antibodies on fluorescent particles increases in proportion to the BA concentrate in specimens, causing reduction in antibody combining sites.

(3) Reaction solution is fed to the immunoreaction channel. BA-abeled bovine serum albumin (BA-BSA) is fixed on thin gold film formed in the immunoreaction channel, so fluorescent-labeled anti-BA antibodies are trapped on thin gold film inversely to the combined BA volume (Step 3). The fluorescent particle volume obtained from the SPF method is automatically converted to the BA concentration by the system to obtain measurement results.

2.3 Points for reaction system development: three types of BA measurement

It is known that major BA of dogs and cats is taurine conjugated cholic acid (CA), chenodeoxycholic acid (CDCA), and deoxycholic acid (DCA) (TCA, TCDCA, TDCA)[7]. Thus this reagent must simultaneously measure these three types of BA to calculate the total volume.

When using one type of anti-BA antibody and one type of BA-BSA, the reactivity against antibodies varies depending on the type of BA, causing the difference of measured values from the results from the enzyme cycling method used in special animal inspection centers.

Using three types of anti-BA antibodies (anti-CA antibody, anti-CDCA antibody, anti-DCA antibody) reacting to three types of BA, namely TCA, TCDCA, TDCA and three types of BA-BSA (CA-BSA, CDCA-BSA, DCA-BSA), we were able to confirm that the inspection results at the same level as the enzyme cycling method's were obtained, and realized simultaneous measurements of three types of BA (Fig. 5).

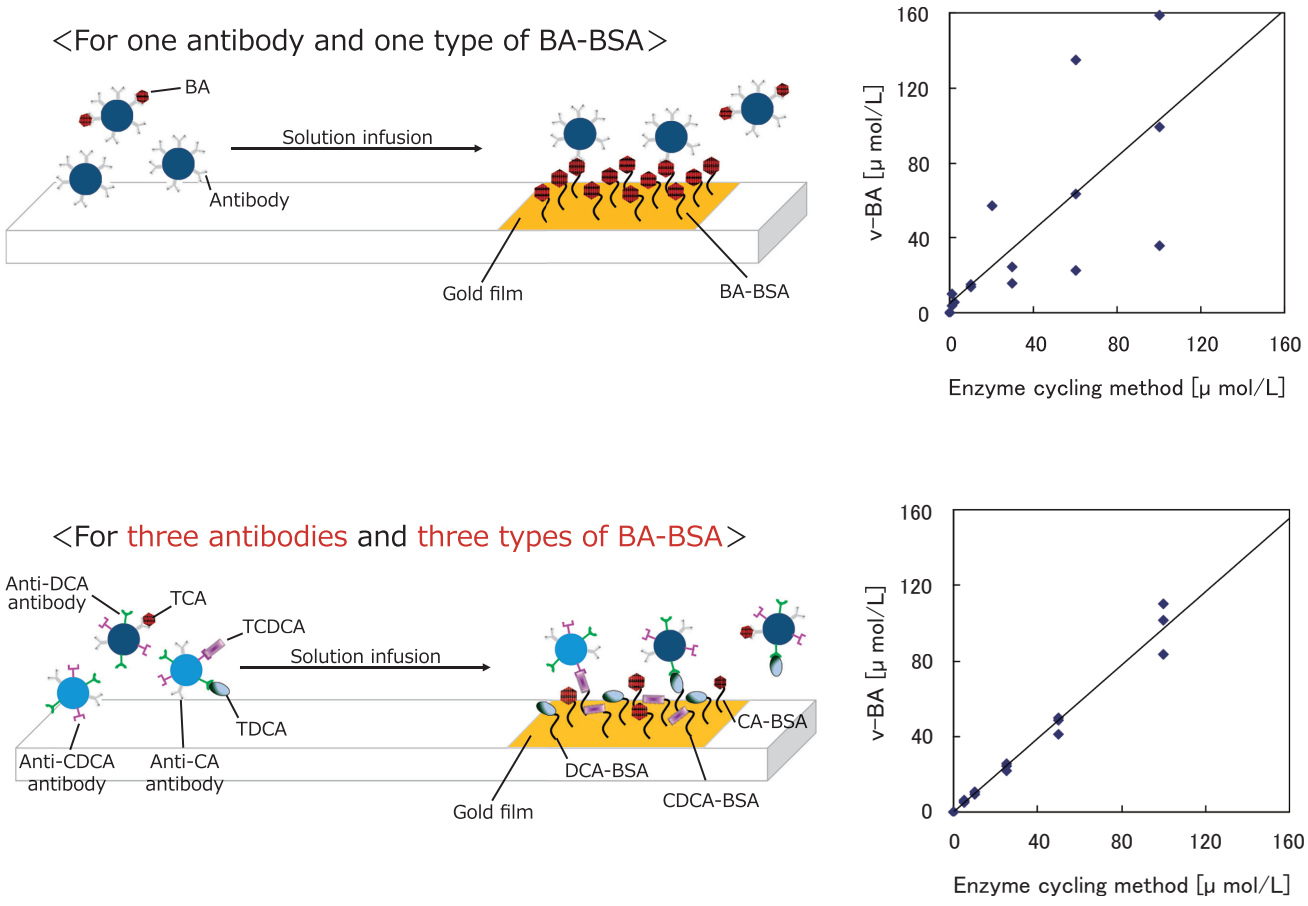

Fig. 5 Effects of three types of anti-bile acid antibodies and bile acid–BSA conjugates

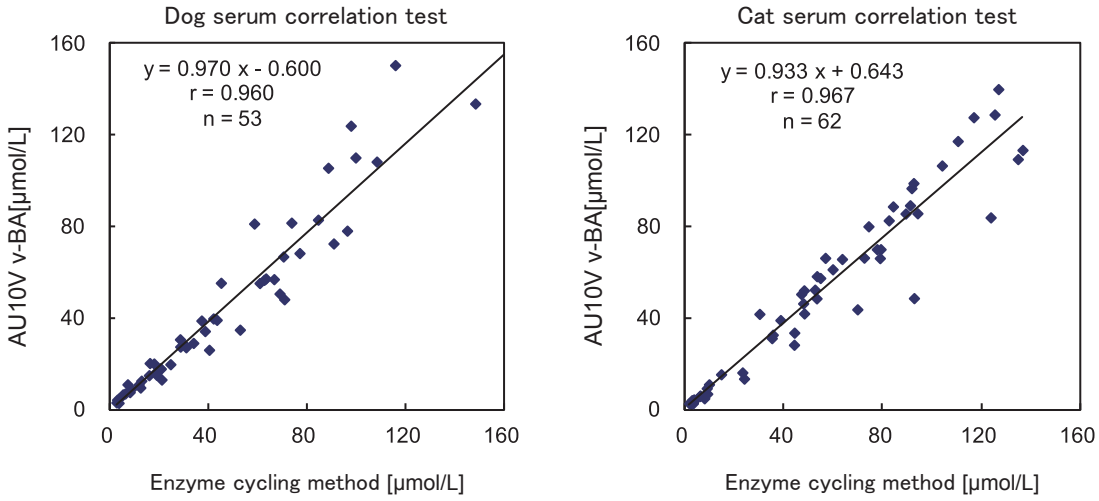

Fig. 6 Correlation between the enzymatic cycling method and FDC v-BA (left: dog serum; right: cat serum)

2.4 Clinical performance

2.4.1 Confirmation of correlation

Aiming to verify the effectiveness of FDC v-BA we have developed this time, we checked the correlativity in dog and cat serums using the enzyme cycling method used in special animal inspection centers as a control method.

The correlation coefficient (r) of FDC v-BA against the enzyme cycling method is 0.960 for dog serum, and 0.967 for cat serum, both indicating correlativity for dogs and cats. Moreover, the slope (a) and intercept (b) of the regression line (y=ax+b; x: enzyme cycling method, y: FDC v-BA) are 0.970 and -0.600 for dog serum, respectively, and 0.933 and 0.643 for cats, respectively. Thus, we confirmed that FDC v-BA brings the BA measurement results for dog and cat serums, same as the enzyme cycling method (Fig. 6). This result shows that an inspection with the same precision as the one that has been outsourced can be performed in hospitals.

2.4.2 Confirmation of precision

We confirmed the simultaneous repeatability when the same sample is repeatedly measured with FDC v-BA. Using three test liquids with the different BA concentration, we measured values 10 times for each liquid. The results are shown in Table 1. Coefficient variation (CV) which is a scale for variation at each concentration level is 3% or less, indicating that this product has a sufficiently high repeatability (quantitativity) as immunoassay reagents.

Table 1 Reproducibility of measurements obtained using FDC v-BA

(Unit: μmol/L)

BA level	Low	Middle	High
1	9.7	34.5	95.6
2	10.5	35.9	101.0
3	9.9	34.9	100.0
4	9.8	34.4	96.2
5	10.2	35.2	98.4
6	9.8	35.0	98.7
7	10.4	36.0	98.2
8	10.0	34.4	101.2
9	9.8	34.9	95.7
10	10.3	35.7	97.9
Ave. (μmol/L)	10.0	35.1	98.3
SD. (μmol/L)	0.3	0.6	2.0
CV. (%)	2.9	1.7	2.1

3. Conclusion

We have developed "FUJI DRI-CHEM IMMUNO AU Cartridge v-BA", a reagent for quantitative immunoassays to complete a fast and simplified diagnostic method for BA.

Technologically, we have established the reaction system simultaneously measuring three types of BA. In the future, we would like to contribute to the improvement of the quality of animal therapy by enriching measurement items to achieve more easy and swift inspections within animal hospitals.

4. Acknowledgment

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