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Development and Validation of a Lateral Flow Device for the Detection of Nicarbazin Contamination in Poultry Feeds

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Concentrations of the coccidiostat nicarbazin as low as 2 mg/kg in feed can result in violative drug residues arising in poultry liver. A lateral flow device (LFD) was developed for the detection of contaminating concentrations of nicarbazin following solvent extraction of poultry feeds. Test results, as determined by both visual and instrumental measurement, are available within minutes. For 22 feed samples, nicarbazin-free and fortified at 2 mg/kg, the % relative inhibition ranged from 0 to 45% and from 53 to 85%, respectively. Nicarbazin contamination at the critical concentration (2 mg/kg) can be determined in all cases providing the sampling is representative. A wide range of feed samples taken at a mill that incorporated nicarbazin into poultry feed were analyzed. Data generated for these samples by both the LFDs and a mass spectrometric method were compared, and a significant correlation was achieved.

KEYWORDS: Lateral flow device; nicarbazin; coccidiostat; poultry; feed

INTRODUCTION

Nicarbazin, an equimolar mixture of 4,4′-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP), has been used globally since 1955 for the prophylactic treatment of coccidiosis in poultry (*1*). Within Europe, under Council Regulation 2205/2001/EC, the license for feed premixes containing nicarbazin as a single active ingredient was withdrawn (*2*). However, in combination with the ionophore narasin, nicarbazin continues to be marketed in the dual-active product Maxiban. Classified as a feed additive and not a veterinary drug, Maxiban is authorized for use in the United Kingdom and Ireland for administration in feed at levels of 40 - 50 mg/kg for broiler chickens but not in layer hens (*3*).

In broilers treated with nicarbazin in their feed, it has been shown that the DNC fraction is more persistent in edible tissues; hence, most analytical methods for the detection of nicarbazin residues are for the DNC component (*4*). To date, no European maximum residue limits (MRLs) have been set for nicarbazin in the edible tissues of broilers. However, the Food and Agricultural Organization/World Health Authority Joint Expert Committee on Food Additives (JECFA) have established an MRL of 200 μg/kg for DNC, as the marker residue, in edible tissue (liver and meat). Residue depletion studies submitted to support the registration of Maxiban have shown that, if correct administration procedures are followed, the mean concentration of DNC residues in poultry liver (n) 6) at 4 days withdrawal is 210 μg/kg (*5*). As a result, a withdrawal period of 5 days is required for broilers treated with Maxiban to ensure that residues are not present in edible tissues. DNC residues have been found in eggs and poultry liver above this level (*6 - 12*). In a report on the surveillance of veterinary drug residues in food in the United Kingdom, nicarbazin residues (DNC) were detected in poultry liver at concentrations ranging between 200 and 3414 μg/kg in 2005 (*13*). The problems associated with having nicarbazin residues in excess of the MRL in poultry produce, however, are more complex than simple nonobservance of sufficient withdrawal periods. A previous study showed that concentrations of nicarbazin of approximately 2 mg/kg in feed may result in DNC levels greater than 200 μg/kg in poultry liver samples (*14*). The cause of residues in the edible tissues of broilers is difficult to assign but can be broadly described as a feed mill or an on-farm problem.

Compliance with the JECFA MRL would be greatly assisted by a simple, fast, cost-effective, and sensitive method to screen for nicarbazin residues in poultry feeds. At present, most methods for the determination of nicarbazin are based on chemical assays that have been developed for application in specialist laboratories (*15 - 18*). However, these methods are

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unsuitable for industrial applications due to cost. As an alternative, rapid methods based on high specificity immunochemical techniques such as lateral flow devices (LFDs) have been proposed and discussed as an alternative for field-based applications for sulfamethazine in pig urine, aflatoxin B_1 in pig feed, and ruminant byproduct material in animal feeds (*19*-*21*).

LFDs are developed from the combination of specific antibodies, colloidal particles (gold, latex, carbon, etc.) as labels, and lateral flow membranes (*22*). The colloidal particles, which are directly or indirectly conjugated to analyte-specific antibody, act as the label for the immunoassay, and the lateral flow membrane operates as the center for separation and targeting of the antibody-bound analyte. As illustrated in **Figure 1**, the LFD is composed of both a control line (C) and a test line (T). At the sample release pad, there are two types of antibody-coated latex particles, control antibodies and specific analyte antibodies. In the LFD, when a sample of the target analyte in solution is placed onto the LFD, the sample commences to flow along the device. The target analyte binds to the target antibody-coated latex particles at the release pad, and the sample migrates across the lateral flow membrane. At the capture window, the T is impregnated with the target antigen protein conjugate. As the target analyte from the sample binds to the target antibody-coated latex particles, most of these latex particles are inhibited from binding to the target antigen protein conjugate at the T and are identified by a decrease or an absence of color reaction at the capture window (**Figure 1a**). This complex of antibody-analyte flows past to the absorbent pad. Hence, if there is no target analyte present within the sample, the target antibody-coated latex particles bind to the target antigen protein conjugate and a color reaction is visible at the T (**Figure 1b**). In concurrence, when a sample is added, the control antibody latex particles traverse the membrane and bind to the control antibody impregnated at the C, which then becomes visible.

LFDs are designed as robust, user friendly, low cost, portable immunoassays that offer a significant reduction in assay time as compared to microtiter plate enzyme immunoassays and other chemical detection methods. They are suitable for the qualitative or semiquantitative detection of specific analytes through the use of a hand-held reader. These are easy to use, low-cost instruments that produce very sensitive and accurate results that allow users to make quick on-site measurements of levels against target reference levels. The principle of the reader is based on the measurement of the correlation of the reflectance value of the T in relation to that of the C. To ensure consistency, it is necessary to employ an internal control mechanism whereby the C is independent of the interactions that are occurring at the T. In addition to surveillance and monitoring programmes of regulatory agencies, these rapid on-site tests may provide a valuable addition to quality control procedures at feed mills and on-farm. Until now, no rapid on-site (farm or mill) method has been available for the detection of nicarbazin cross-contamination in animal feeds.

In this paper, the development of a simple, portable, and rapid LFD for on-site testing for the DNC component of nicarbazin in poultry feeds is described for application on farm or at feed mills. The method has been applied to feed samples contaminated with nicarbazin and was found to be most appropriate.

MATERIALS AND METHODS

Safety. All chemicals should be used under the conditions stated in the Control of Substances Hazard to Health (COSHH) assessments: http://www.coshh-essentials.org.uk.

Materials. F-Nitrosuccinanilic acid (NSA), human serum albumin (HSA), apotransferrin (TF), bovine thyroglobulin (BTG), glutamic acid γ-(*p*-nitroanilide) (GAN), carbonyldiimidazole, and Tween 20 were obtained from Sigma Aldrich (Poole, United Kingdom). N-Succinyl-L-alanyl-L-alanine 4-nitroanilide (SAN) was obtained from Fluka (Gillingham, United Kingdom). 4,4-Dinitrocarboanilide (DNC) [N,N′-Bis(4-nitrophenyl)urea] was obtained from Aldrich (Gillingham, United Kingdom). Sodium sulfate, sodium chloride (general purpose reagent), ammonium sulfate, and all solvents of analytical grade [dimethylformamide (DMF), acetone, and methanol] were purchased from BDH (Poole, United Kingdom). Anhydrous sodium acetate was obtained from VWR International (Lutterworth, United Kingdom).

LFD Components. Blue latex microspheres (0.43 μm) were obtained from Bangs Laboratories (Fishers, IN). The quick release pads (8 mm wide), membrane 120, membrane 135, membrane 180, and glass fiber sample pads were purchased from Millipore Corp. (Bedford, MA). The large pore direct cast membranes Prima 40 (PR40), Prima 60 (PR60), and Prima 85 (PR85) were supplied by Whatman (Dassel, Germany). For all membrane types, the number of the membrane indicates the nominal flow rate, i.e., the flow time in seconds across 4 cm of membrane. The anti-rabbit IgG (whole molecule) antibody produced in goat was purchased from Sigma-Aldrich.

Production and Characterization of Polyclonal Antibody for Nicarbazin. For the production of polyclonal antibodies, rabbits were immunized with two DNC mimic protein conjugates: NSA-HSA and SAN-HSA. The chemical synthesis of the immunogen, the immunization process and antibody titer determination, and the assessment of antibody sensitivity and specificity by enzyme linked immunoabsorbent assays (ELISA) have been described in detail in a previous paper (*23*). Each antibody was purified by precipitation with saturated ammonium sulfate, and the protein concentration was determined by measurement of absorbance at 280 nm.

Preparation of LFD Protein Conjugates. Two conjugates, GAN-TF and GAN-BTG, were produced for the development of the LFDs as follows: GAN (20 mg) was dissolved in 2 mL of dry DMF (dried over sodium sulfate), and 2 mL of dry acetone (dried by distillation) was added. Carbonyldiimidazole (60 mg) was dissolved in 4 mL of dry acetone and added to the solution of GAN. The mixture was stirred at room temperature for 4 h. The solvent was evaporated at room temperature under a stream of nitrogen to leave approximately 1100 μL of DMF containing the activated GAN.

TF (50 mg) was dissolved in 4 mL of carbonate buffer, pH 9.5, and 1 mL of DMF. The activated GAN solution (550 μL) was added to the TF solution. BTG (50 mg) was dissolved in 4 mL of carbonate buffer, pH 9.5, and 1 mL of DMF. The activated GAN solution (275 μL) was added to the BTG solution. Both mixtures were allowed to incubate for 48 h at 4 °C. The conjugates were purified by dialysis against 0.15 M saline solution (3 × 4 L).

Latex Labeling of the Antibody. Each antibody (2.0 mg/mL) was bound independently to blue latex microspheres using a process of passive adsorption to produce two stock solutions of antibody-coated latex particles in suspension. The latex and antibody were incubated under continual rotation for 2 h at 35 °C, blocked with a simple blocking solution for 30 min, washed twice using borate buffer, and finally resuspended in storage buffer. Each stock solution of antibody-coated latex particles was diluted one in 10 with storage buffer and applied onto woven polyester release pads (8 mm wide) via saturation using an immersion technique. The release pads were then air-dried on a stainless steel pan at 35 °C to produce a stable reservoir for antibody-coated latex release, prior to incorporation into assembled devices.

Application of the Protein Conjugate and Control Antibody to the Membrane. Each protein conjugate (GAN-TF or GAN-BTG) and goat anti-rabbit IgG antibody (0.2 mg/mL) was applied as the T and C, respectively, onto each membrane using a Biodot XYZ reagent dispenser (Biojet, Chichester, United Kingdom). The dispenser applied combinations of the protein conjugate and control antibody reagents linearly and parallel to each other, at a distance 5 mm apart, on a 35 cm strip of membrane. The membrane was air-dried, immersed in membrane-blocking buffer, rinsed, and air-dried.

A range of concentrations of both protein conjugates, GAN-TF or GAN-BTG, were examined in permutation with various nitrocellulose

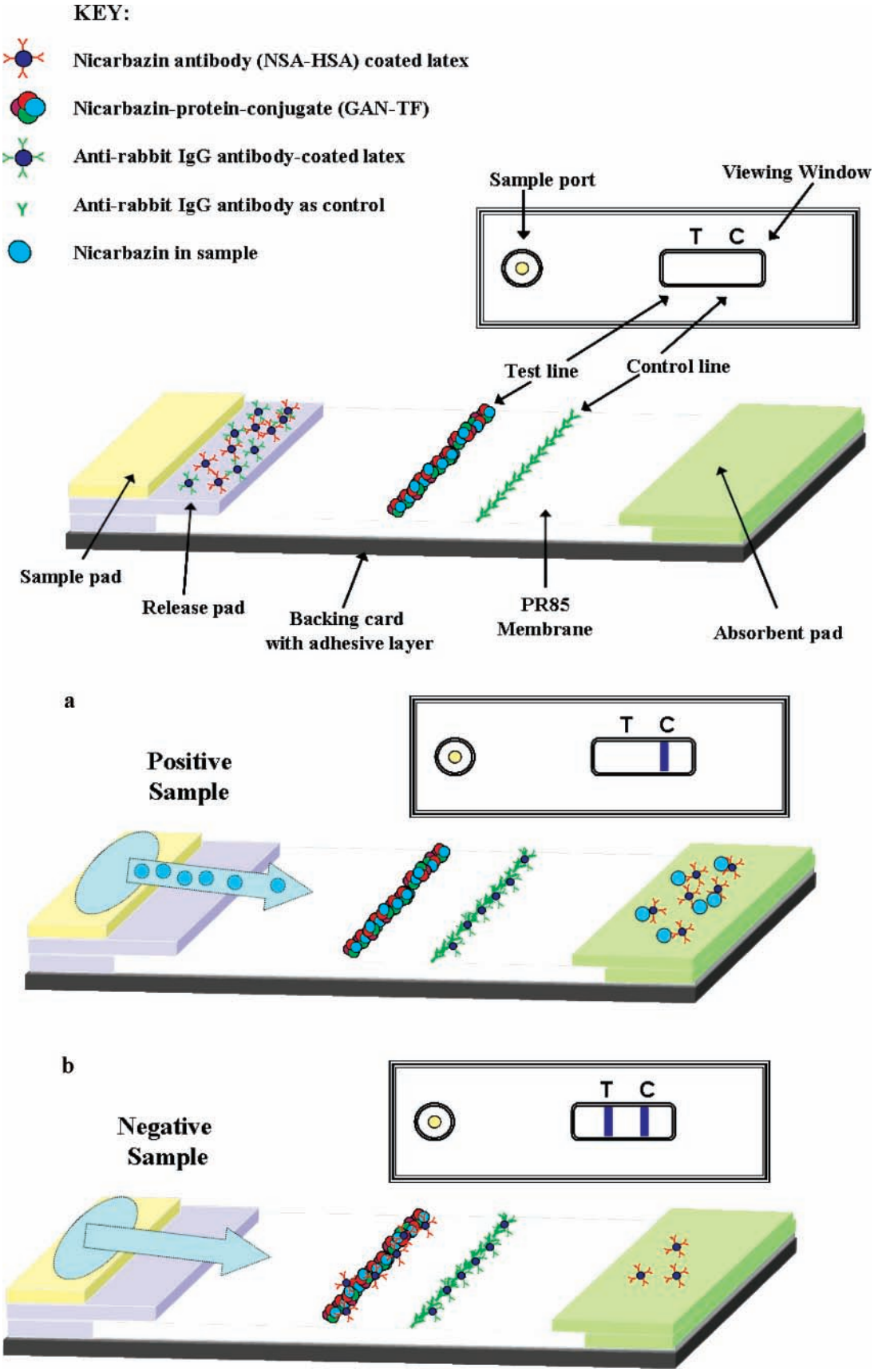

Figure 1. Diagrammatic cross-section of LFD components.

membranes (120, 135, 180, PR40, PR60, and PR85) varying in manufacturer, thickness, pore size, and nominal flow rate using a wet assay format. In the wet assay format, the membrane adhered to the backing card just overlying at one end. An absorbent pad was attached at the opposite end. The membrane was dipped into a mixture of control and NSA-HSA or SAN-HSA antibody-coated latex solution in a 96

well ELISA plate. Checkerboard evaluation of each assay format using inhibition with 0, 0.1, 1.0, 10, and 100 ng/mL of DNC standard was performed.

Assembly of LFDs. An illustration of the assembled components is shown in **Figure 1**. Essentially, the membrane was adhered to a backing card and a cellulose fiber absorbent pad was attached. The antibody-coated latex release pad was then secured to the backing card just overlying the membrane. Similarly, a glass fiber sample pad was attached to the backing card just overlying the antibody-coated latex release pad. The assembled devices were encased into plastic housings for assessment with a range of DNC standards in PBST buffer (pH 7.2).

Extraction Protocol. A simple extraction protocol was devised that could be performed on farm or at a feed mill to examine the effects that poultry feed samples would have on the performance of the LFDs. Solvent extraction (10 mL of methanol) was used to extract nicarbazin from poultry feed samples (10 g). The feed sample was ground in the solvent using a spatula, and the mixture was shaken vigorously for 30 s. The mixture was allowed to settle for 10 min. The supernatant (0.5 mL) was diluted 1/10 in diluting buffer [4.5 mL, pH 7.2, phosphate buffer saline with tween (PBST)] and mixed.

LFD Assessment and Validation for Poultry Feed Samples. A working prototype was produced for determination of nicarbazin levels in the range 0 - 2 mg/kg in poultry feed. The device was optimized for DNC standards in the range 0 - 150 ng/mL in PBST buffer (pH 7.2) with 10% methanol. Control poultry feeds (22 samples) were obtained from feed mills where Maxiban was not used as an additive in feed manufacture. Two subsamples (10 g) were taken from each feed. One (positive material) was fortified with 2 mg/kg of nicarbazin (the recognized threshold level to trigger contamination). The second (negative material) remained free from nicarbazin addition. Both samples were subjected to the sample extraction protocol described earlier. A portion (80 μL) of the resulting extract was applied onto the LFDs, and the reflectance values for the reference area (R), the C, and the T were measured after 10 min via a hand-held reader (Chromato-reader Type 1, Otsuka Electronics Co., Osaka, Japan). Statistical analysis was performed.

Trial Investigation of Poultry Feed Samples at Feed Mill. A field study was conducted to test the performance of the devices for utilization at or close to the feed production line. A 3 ton batch of nicarbazin-medicated feed at 50 mg/kg was manufactured. This was followed by the production of five sequential 3 ton batches of nonmedicated feed. Five samples of approximately 200 g each for each batch were taken from each of the five batches at regular time intervals as the feed passed by the sample hatch prior to the prepress bin. Samples were analyzed using the LFD before and after thorough mixing of the sample using a PT 100 sample divider (Retsch UK Ltd., Leeds, United Kingdom). Samples were also analyzed using a previously developed liquid chromatographic - electrospray mass spectrometry (LC-MS/MS) method (*15*).

RESULTS AND DISCUSSION

Antibody Production. Polyclonal antibodies were raised following the immunization of rabbits with two DNC mimic protein conjugates: NSA-HSA and SAN-HSA. Both polyclonal sera from the rabbits exhibited significant antibody titers and showed a high degree of specificity and sensitivity for DNC when evaluated using a competitive ELISA. Dose - response curves for DNC were obtained ranging from 0 to 100 ng/mL, and these have been illustrated in a previous publication (*23*).

Preliminary Evaluation of LFDs. In the wet assay format, the antibody-coated latex solution was drawn up the membrane and behaved in a similar fashion to the final assembled device but without the preparation of a release pad. This was a quick and simple method that enabled combinations of antibody-coated latex and membranes to be assessed simultaneously.

The first stage was to ensure that the antibody coating of the latex microspheres had been achieved and that these antibody-coated latex microspheres could in turn bind to the DNC mimic protein conjugate bound at the T. This was established by the appearance of the T on the membrane following the application of a negative sample in PBST buffer (pH 7.2) (**Figure 2**). Ts were produced on all of the membranes evaluated although the lines were substantially weaker in intensity when compared to the C. However, membranes 120, 135, and 180 produced Ts of lower intensity as compared to membranes PR40, PR60, and PR85. This effect was more noticeable with the NSA-HSA antibody. This finding may indicate that for this application, these membranes (120, 135, and 180) may be less efficient in the binding of the protein conjugate reducing the amount of antibody that could attach. It might also be suggested that the slower nominal flow rates of these membranes may deter the interaction of the antibody-coated latex with the T protein conjugate occurring. Nevertheless, the antibody appeared to have conjugated well to the standard blue latex microspheres and exhibited a good flow performance. For both protein conjugates applied at the same concentration, the T appearance and intensity of the GAN-TF T, for a negative response, was better on comparison with that of the GAN-BTG T.

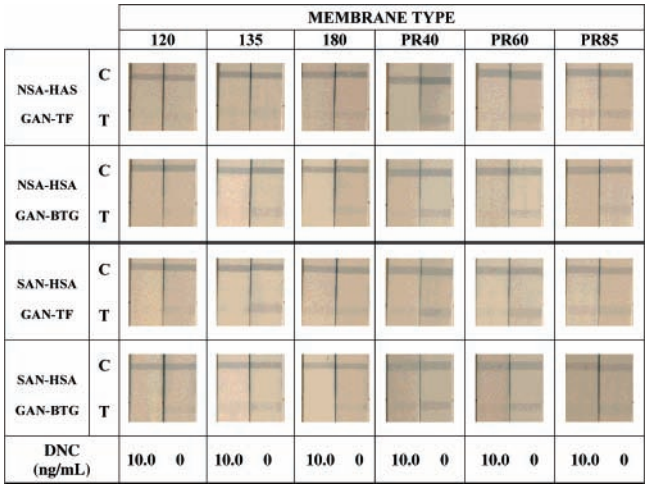

Figure 2. Wet assay analysis of each membrane type for both protein conjugates.

Equally important, inhibition of the antibody coated-latex binding to the protein conjugate at the T was produced on the application of a 10 ng/mL DNC standard in PBST buffer (pH 7.2). Preliminary results established that a number of the LFD prototypes constructed were capable of detecting DNC. However, the interaction of the antibody with the GAN-TF protein conjugate displayed slightly greater inhibition for a positive sample as compared to that of the GAN-BTG protein conjugate.

In combination with each of the antibody-coated latex particles, membranes PR60 and PR85 were further assessed, following the addition of the release pad and full assembly of the LFD, with DNC standards in PBST buffer (pH 7.2). From the assembled devices, NSA-HSA antibody-coated latex produced Ts of darker intensity as compared to that of the SAN-HSA on the application of a negative sample (**Figure 3**). As this difference in intensity was not as significant with the wet assay format, it could be deduced that the release of SAN-HSA from this release pad was not as efficient as for NSA-HSA.

The assembled devices with either the PR60 or the PR85 membrane with NSA-HSA antibody-coated latex and GAN-TF protein conjugate were assessed using a range of DNC standards in PBST buffer (pH 7.2) (**Figure 3**). Membrane PR85 displayed the better line development (**Figure 4**). Lines of

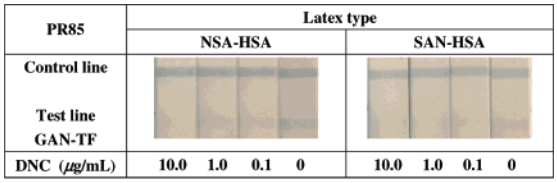

Figure 3. Latex comparison in assembled devices using nitrocellulose membrane type PR85.

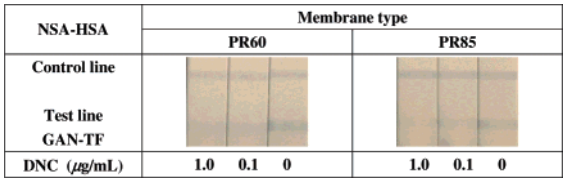

Figure 4. Membrane comparison in assembled devices using latex-coated antibody NSA-HSA.

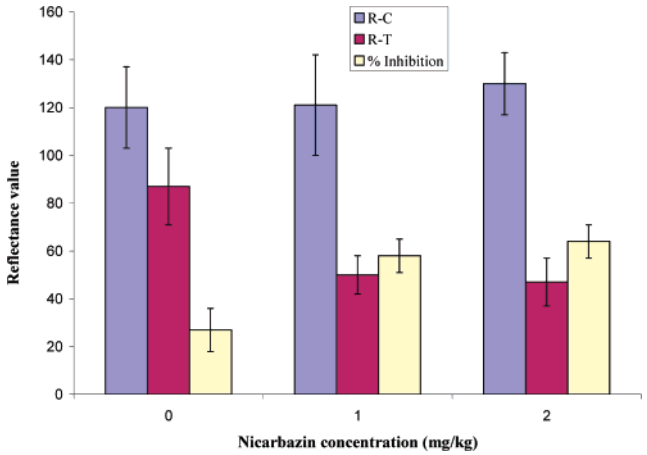

Figure 5. Relationship between the concentration of nicarbazin within a sample and the inhibition of T development for validation samples.

similar intensity and consistency were produced for both the Cs and the Ts within an acceptable development time for the negative standard, and inhibition of this T with DNC was observed. In contrast, a broader, diffuse T as compared to that of the C was apparent using the PR60 membrane. Hence, the membrane flow characteristics may also be a governing factor for visual appearance of the T. The faster flow rate of the PR60 may allow greater lateral spread of the protein conjugate when applied by reagent dispenser, producing a more diffuse line.

From this preliminary evaluation of the LFD, the final components selected were as follows: C (goat anti-rabbit IgG), 0.2 mg/mL; T (GAN-TF), 0.1 mg/mL; polyclonal NSA-HSA antibody, 2.0 mg/mL; and goat anti-rabbit IgG antibody, 2.0 mg/mL.

Assessment and Validation of LFD Prototypes for Poultry Feed Samples. On assessment of the prototypes using nicarbazin-free poultry feed extracts, it was found that it was not possible to fully inhibit the T over the desired sensitivity range. Investigations suggested that the sizable inhibitory affect upon T development may be due to the influence of the constituents of poultry feed and extraction solvent; that is, a significant matrix effect was evident. Nevertheless, determination of the extent of T inhibition was achieved by the use of a hand-held portable reader.

A validation study was performed using the final prototypes on feed samples. This working prototype was produced for determination of nicarbazin levels in the range 0 - 2 mg/kg in poultry feed. The amount of 2 mg/kg was determined as the desired cutoff level. The JECFA MRL for poultry liver is 200 μg DNC per kg, which has been determined experimentally to be brought about by nicarbazin in feed at a level of approximately 2.5 mg/kg, hence, the desired cutoff.

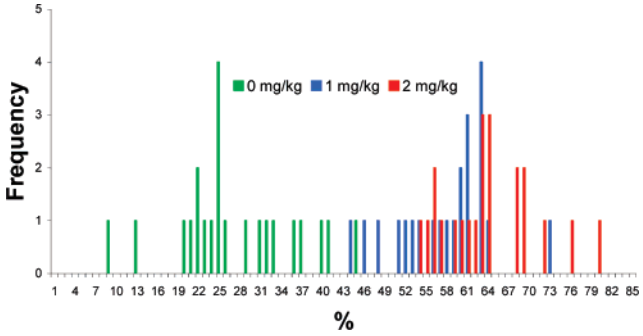

Figure 6. Relationship between the concentration of nicarbazin in validation feed samples and the % inhibition of the T as recorded by the hand-held reader.

Table 1. Analysis of Nicarbazin-Fortified Feed by LFD

nicarbazin concentration in fortified feed (mg/kg)	range of % inhibition of the T for 22 poultry feed samples
0	8.9–44.8
1	44.0–64.4
2	53.5–80.3

The device was checked for DNC standards in the range 0 - 150 ng/mL in PBST buffer (pH 7.2) with the addition of methanol (10%), thus equating to the 10-fold dilution as produced by the extraction procedure (assuming approximately 100% efficiency of DNC extraction by the solvent). The reader measured the reflectance values of T, C, and R (the background area between the two lines) from which the % relative inhibition of the T to the C could be calculated using this equation: 100 - (R - T/R - C × 100). The calculated % relative inhibitions for a negative sample and a sample fortified with 150 ng/mL were 1 and 59%, respectively.

The prototype devices were validated on a range of fortified and manufactured feed samples using the extraction protocol. Feed, fortified with nicarbazin, was analyzed using the prototype LFD, in combination with the reader, to measure T, C, and R. **Figure 5** shows the calculated results for the range of fortified feed samples including data for the intensity of C (R - C), T (R - T), and % relative inhibition. Feed samples fortified with nicarbazin at 2 mg/kg were clearly identified from nicarbazin-free feed samples. The results demonstrated that there was a relationship between the feed nicarbazin concentration and the inhibition of T development. Hence, the nicarbazin content of an unknown sample may be partially quantified based on the resulting level of inhibition measured. The greater the % relative inhibition is, the higher the concentration of nicarbazin present within the sample is.

Correspondingly, **Table 1** and **Figure 6** display the range and frequency of the % relative inhibition calculated for each of the fortified levels of nicarbazin in the 22 validation feed samples. The nicarbazin-free feed samples showed the greatest variation in % relative inhibition. In the case of these same samples fortified at 1 mg/kg, in only one case did the % inhibition obtained fall within the range of negative samples. In the samples tested with the 2 mg/kg level of fortification, there was no overlap in the range of % inhibition for these samples and for nicarbazin-free samples.

Table 2. Comparison of LFD and LC-MS/MS Analysis for Nicarbazin-Fortified Feed

	batch 2		
sample	nonmixed feed tested by LFD (% inhibition of T)	mixed feed tested by LFD (% inhibition of T)	confirmatory analysis by LC-MS/MS (ppm)
1	43.4	61.7	7.6
2	80.3	76.1	36.1
3	36.0	42.4	0.3
4	44.6	42.8	0.1
5	29.4	38.6	0.4

Statistical analysis was performed using a standard *t* test. *T* tests were carried out to examine whether there are significant differences between each of the fortified levels. It was revealed that there are significant differences between the 0 and 1 mg/kg (t) - 12.737, p) 0.241), the 0 and 2 mg/kg (t) - 15.300, p) 0.198), and the 1 and 2 mg/kg (t) - 2.961, p) 0.870) nicarbazin levels. From this data, it was deduced that a 53% relative inhibition threshold level would identify samples most likely to contain nicarbazin at 2 mg/kg. At this threshold level, the percentage probability of obtaining a false positive of 0 mg/kg was 0% and of 1 mg/kg was 24%; however, the probability of obtaining a false negative was 0%.

Trial of Final Prototypes on Manufactured Feed Samples. Medicated feed (batch 1) tested with the LFD resulted in 82.4% relative inhibition of T development. The concentration of nicarbazin in this sample was confirmed by LC-MS/MS to be 31.1 mg/kg. For the subsequent batch of feed produced (batch 2), the first sample (*1*) analyzed by the LFD without mixing and after mixing using the sample divider produced % relative inhibitions of 43.4 (i.e., below the 53% threshold) and 61.7% (i.e., above the 53% threshold), respectively (**Table 2**). Nicarbazin at a concentration of 7.6 mg/kg was confirmed by LC-MS/MS. This result indicates that analysis based on a small subsample of feed, without prior mixing of the larger sample, may result in inaccurate results for nicarbazin content in feed. LFD analysis of the second sample (*2*) of this batch provided 80 and 76% inhibition for nonmixed and mixed feed samples. Nicarbazin at a concentration of 36 mg/kg was confirmed by LC-MS/MS. Samples 3 - 5 were all deemed to have nicarbazin below 2 mg/kg by the LFD procedure, and LC-MS/MS confirmed these results to be accurate. Four more consecutive batches of feed were tested as described but neither the LFD nor the LC-MS/MS detected the presence of nicarbazin at concentrations greater than 2 mg/kg.

These results indicate that whether the sample is taken at the start, middle, or end of a batch can strongly influence the outcome obtained. To ensure representative sampling of the feed and deliver a more reliable result using the LFD, it is important to analyze a number of subsamples at regular time intervals on the feed production line. The results from the feed mill study also clearly demonstrate that the prototype LFDs could be utilized at the feed production line to ensure that contaminated feed can be identified quickly and reliably.

With increased public awareness of food safety issues, good manufacturing practices at poultry feed mills, and good poultry farm management, suitable tools for monitoring the content of nicarbazin in feed at every stage of poultry production are required. Many of the test methods for nicarbazin are high technology laboratory-based tests that may be impractical and time-consuming when a feed manufacturer or farmer wishes to test the feed for nicarbazin contamination at production or on delivery.

The LFD developed here shows how the principle of lateral flow technology can be applied to the detection of nicarbazin in feeds at concentrations at or above 2 mg/kg and offers a simple and effective way of assessing nicarbazin contamination in the field. By using this rapid diagnostic test, implications of nicarbazin contamination due to feed mill, transportation, and farming procedures can be quickly and easily identified at a reasonable cost. Personnel assessing nicarbazin contamination using this method would only need a moderate degree of training, and as no subjective interpretation of the results is required, bias and individual variation may be eliminated. This enables the developed LFD devices to be used by a range of differently skilled workers under varying conditions while providing consistent and reliable results. The provision of this cheap and easy to use tool will enable feed manufacturers and farmers to incorporate this into their quality assurance procedures.

ABBREVIATIONS USED

BTG, bovine thyroglobulin; C, control line; DMF, dimethylformamide; DNC, 4,4′-dinitrocarbanilide; LC-MS/MS, liquid chromatographic - electrospray mass spectrometric; JEFCA, Joint Expert Committee on Food Additives; GAN, glutamic acid γ-(*p*-nitroanilide); HDP, 2-hydroxy-4,6-dimethylpyrimidine; HSA, human serum albumin; LFD, lateral flow device; MRL, maximum residue limit; NSA, F-nitrosuccinanilic acid; PBST, phosphate buffer saline with tween; PR, prima; R, reference area; SAN, N-succinyl-L-alanyl-L-alanine 4-nitroanilide; T, test line; TF, apotransferrin.

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