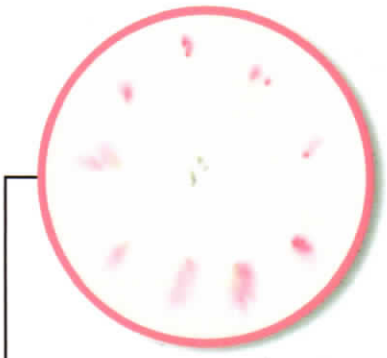
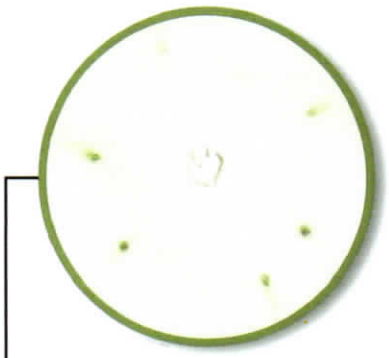
Microtracer COLOUR Card

ELANCO ANIMAL HEALTH™

This colour reference chart has been provided as a service to our customers.

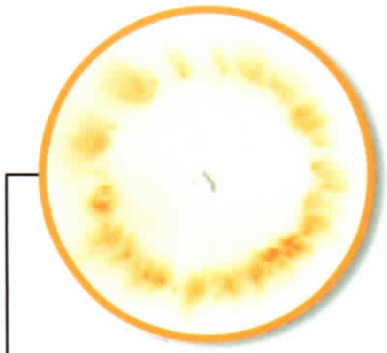


ELANCO COBAN/RUMENSIN
50 ppm**
Microtracer FS Red #3/Natural Yellow
Developer: 50% alcohol with ammonia*

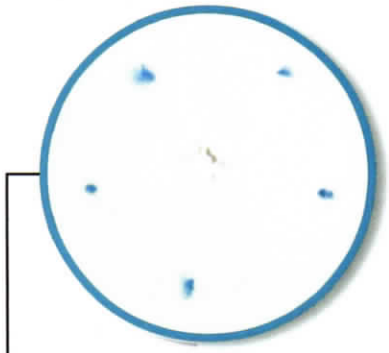


ELANCO MONTEBAN
ELANCO MAXIBAN
20 ppm**
Microtracer FS Natural Green
Developer: 50% alcohol with ammonia*

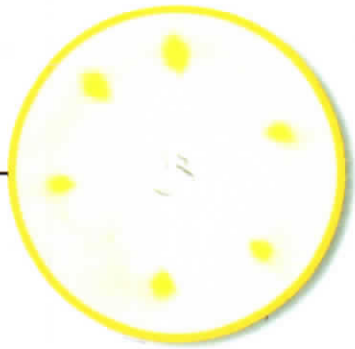
COXISTAC
Microtracer FS Violet (Red #3/Blue #1)
Developer: 50% alcohol with ammonia



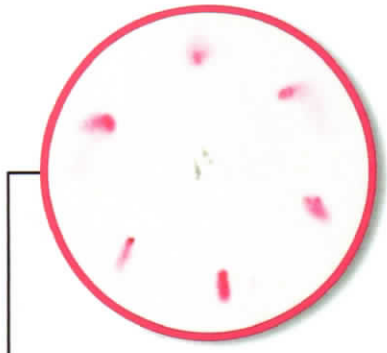
MONENSIN PREMIX
Microtracer RF Gold Lake
Developer: 7% Sodium Carbonate in water



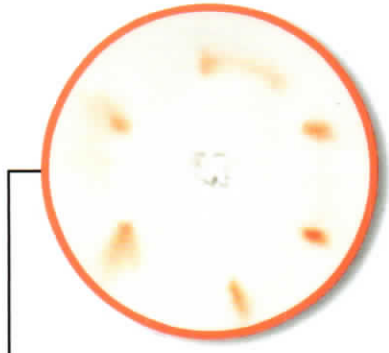
NICARB
Microtracer FS Blue #2
Developer: Deionized Water



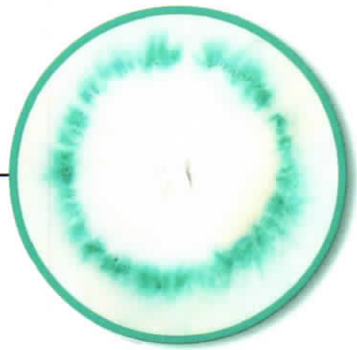
SACOX
Microtracer F Natural Yellow
Developer: 100% alcohol



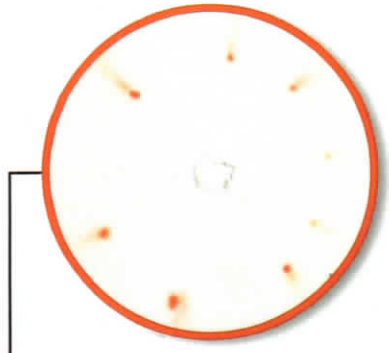
SALINOMYCIN 60 PREMIX
Microtracer F Dark Red
Developer: Deionized Water



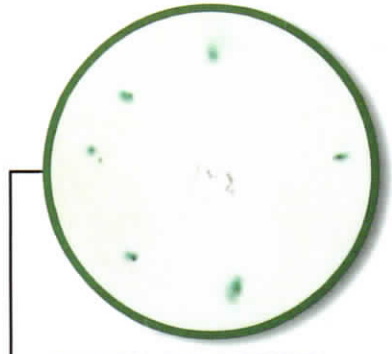
STENOROL
Microtracer FS Orange (Yellow #6)
Developer: Deionized Water



SUPER CHLOR 250
Microtracer RF Green Lake
Developer: 7% Sodium Carbonate in water



ZINPRO AVAILAMINS
Microtracer F Red #40/Yellow #5
Developer: 50% alcohol with ammonia



ZINPRO 100 PREMIX
Microtracer F Green #3/Yellow #6
Developer: 50% alcohol with ammonia

Mason Jar Technique

Materials

1. Scale suitable for weighing 65 grams of feed
2. Whatman No. 1 filter paper, 7.0cm diameter
3. Coffee mill or Osterizer
4. Developing solution (70% ethanol/30% water with 2-5 drops of concentrated ammonium hydroxide per 100ml)
5. Pint mason jar
6. Special annular magnetic cap
7. Hot plate or mug warmer

Test Method

1. Turn on the hot plate (250-300°F) or mug warmer.
2. Weigh about 65 grams of feed (grind feed if in pellet form).
3. Transfer the feed to the mason jar.
4. Insert filter paper into the magnetic cap and close jar.

5. Shake jar so that all of the sample contacts filter paper within cap. Shake for at least 1 minute.

6. Tap the cap to remove any feed that has adhered to the filter paper. Remove the cap and place horizontally with the filter paper visible.
7. Transfer 10-15 drops of the ethanol developing solution to the center of the filter paper. Let the alcohol diffuse outward through the ring of trapped microtracer particles.
8. Remove the filter paper from the cap using a spatula or small knife. Allow the alcohol to evaporate for at least 30 seconds. Then place the filter paper on the heated hot plate or mug warmer.
9. Observe the color on the filter paper for qualitative identification.

Mircrotracer Reg. T.M. Micro Tracers, Inc

POSITION STATEMENT

ZINPRO
PERFORMANCE MINERALS®

Method of Identifying Zinpro Products in Premixes, Feeds

Purpose of Using Microtracers

To assure that Zinpro Corporation customers are receiving Zinpro products in their feeds as they have specified.

Procedures

Below is a step-by-step description of how the Microtracer Rotary Detector functions. Please refer to Zinpro Tracer Sampling and Analysis Guidelines (Position Statement G-1012) for a detailed explanation of procedures for sampling, analysis, interpretation of results and follow-up communications.

Recommendation

We recommend that premix, supplement and/or concentrate feed samples be sent directly to Zinpro Corporation for tracer analysis.

Mailing Address:
Zinpro Corporation
Attn: Quality Control Manager
6375 415th Street
North Branch, MN 55056

Microtracer Rotary Detector Technique




Materials Required

1. Scale suitable for weighing 500 grams of feed.
2. Whatman No. 1 filter paper, 9.0 cm in diameter.
3. Coffee mill or Osterizer (for grinding pelleted product).
4. Developing solution.
5. Mug warmer or hot plate.
6. Microtracer Rotary Detector.

Testing Procedure

1. Turn on mug warmer or hot plate (250-300°F or 120-150°C).
2. Weigh 500 grams of feed (grind if in pelleted form).
3. Place filter paper on spindle of rotary magnet.
4. Turn on Rotary Detector.
5. Transfer sample of feed to the Rotary Detector's top hopper.
6. After hopper is empty, turn off Rotary Detector. Remove top hopper.
7. Add 10-15 drops of developing solution to the center of the filter paper. Let it diffuse outward through the ring of trapped microtracer particles.
8. Remove filter paper from rotary magnet with spatula or small knife.
9. Place filter paper on mug warmer or hot plate. Check filter paper for colored spots of microtracer.

Zinpro Corporation
10400 Viking Dr. | Suite 240 | Eden Prairie, MN 55344 | USA
800.445.6145 • 952.983.4000 • fax 952.944.2749 • www.zinpro.com

Method of Identifying Coxistac® 6% and Posistac® 6% Premixes in Finished Feeds

Electric rotary detector.

MATERIALS REQUIRED

1. Scale suitable for weighing 500 grams of feed.
2. Whatman No. 1 filter paper, 9.0cm diameter.
3. Coffee mill or Osterizer.
4. Developing solution (70% ethanol/30% water with 2-5 drops of concentrated ammonium hydroxide per 100 mL).
5. Mug warmer or hot plate.
6. Microtracer® Rotary Detector.

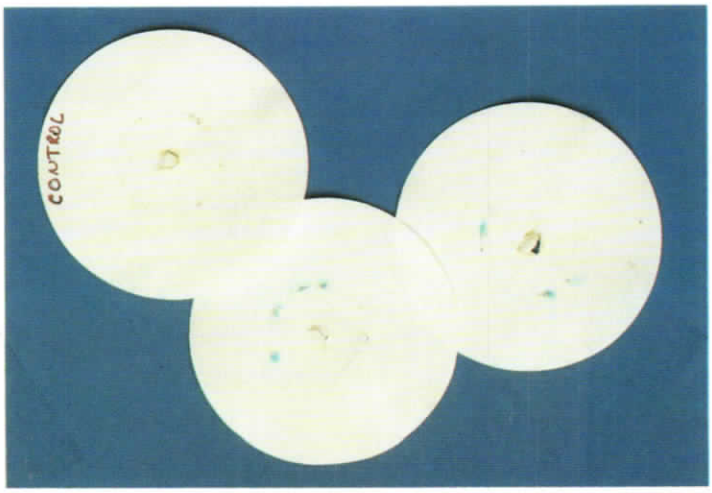
A negative result with the tracer test does not guarantee the absence of Coxistac* 6% or Posistac* 6% in finished feed.

If a quantitative measure of the level of salinomycin is required, contact your Pfizer Animal Health Sales Representative.

As a good manufacturing practice, **avoid** manufacturing feeds for turkeys, horses and dogs directly after broiler, hog or beef cattle feed containing Coxistac* or Posistac*.

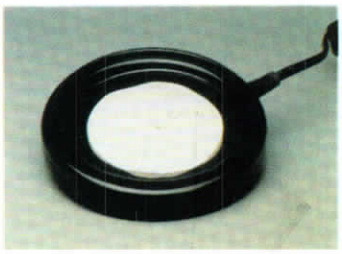
METHOD

1. Turn on the hot plate (250 to 300°F or 120 to 150°C) or mug warmer.
2. Weigh 500 grams of feed (grind feed if in pellet form).
3. Place filter paper on the spindle of the rotary magnet.
4. Turn on the Rotary Detector.
5. Transfer the sample of feed to the top hopper of the Rotary Detector.
6. After the hopper is empty, turn off the Rotary Detector. Remove the top hopper.
7. Add 10-15 drops of the ethanol developing solution to the center of the filter paper. Let the alcohol diffuse outward through the ring of trapped particles.
8. Remove the filter paper from the rotary magnet with a spatula or small knife. Allow the alcohol to evaporate for at least 30 seconds.



A daily positive control test must be performed on feeds manufactured that day and known to contain Coxistac* 6% or Posistac* 6% premix.

N.B. Following the recovery of a positive sample, the equipment should be cleaned and dusted to avoid contamination and false positive test.



9. Place the filter paper on the heated hot plate or mug warmer. Check the filter paper for coloured spots of microtracer for qualitative identification.

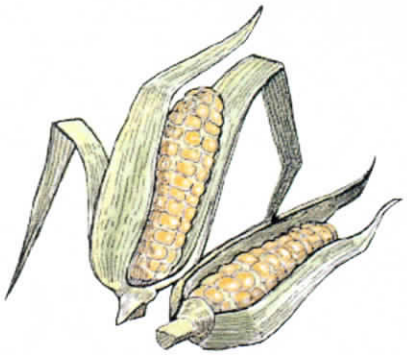
Animal Health Division
Pfizer Canada Inc.
Montreal H9R 4V2

Pfizer Animal Health

* Registered trademark of Pfizer Inc. Pfizer Canada Inc. is a Registered User.

® Registered Trademark of Micro-Tracer Inc. San Francisco, California.

The use of Microtracers™ F (colored uniformly sized iron particles) in coding the presence of coccidiostats in poultry feeds. Practical implications.



NUTRITION

David A. Eisenberg

In a 1993 article, Zootecnica reported on the potential toxicity of various coccidiostats and on their potential to lead to toxicity or illegal tissue residues in poultry meat. This paper also explained how Microtracers F (colored uniformly sized iron particles) had been used to reduce the incidence of feed manufacturing mistakes leading to such problems. While the paper described Microtracers and applicable test procedures, it did not discuss the details involved in establishing a quality assurance program nor did it provide any quantitative data from feedmills where tracers were employed on an ongoing basis. This paper provides this information and discusses the implications of such testing to concerns expected to impact the feed manufacturing industry in the future.

Poultry feed manufacturing

In the USA, nearly all broiler feed is manufactured at feedmills where only poultry feed is produced. At some mills, production may not be limited to broiler feed but may also include turkey and breeder feeds. These feedmills often operate nearly 24 hours a day 7 days per week with production capacities of 100 tons/hour or more. Production runs for individual formulas are usually longer than at commercial feedmills manufacturing feeds for many species. The number of formulation changes may also be fewer.

Even though such mills are highly automated, mistakes can and do occur. The coccidiostat may be loaded manually into the wrong bin in a computerized micro-addition system, or someone may make an error in programming the computer or the system itself may malfunction. Feed may bridge and not flow properly in conveyer or elevator legs or in holding bins supposed to be empty.

Discharge gates on a mixer may leak allowing feed ingredients in a second batch to contaminate an earlier batch not discharged from a surge bin below the mixer.

While the incidence of such errors is very low, the costs associated may be great and the anxiety may be prohibitive unless the feed manufacturer can implement a "real time" quality assurance program where feed is tested before it leaves the feedmill. This testing must be designed to reduce the incidence of manufacturing errors to as nearly zero as is possible.

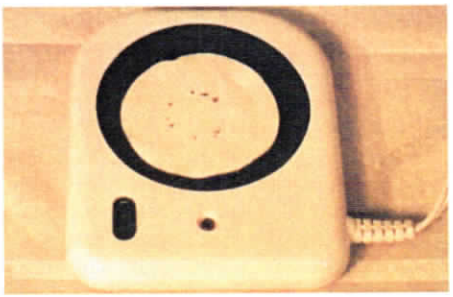
Micro-Tracer "real time" quality assurance of poultry feed

When a Microtracer is included as an ingredient in a coccidiostat premix, it often serves two purposes. In addition to providing feed manufacturers a "quick test" for the medication in their final feeds, it may also identify the premix and feed as proprietary. The tracer is usually formulated to yield 63 particles per 500 grams of feed. Tracer recovery is typically 80% from mash feeds and 65% from pelleted feeds so the feed manufacturer actually expects about 40 tracer particles from analysis of 500 grams of a pelleted feed. The likelihood of finding no tracer when one is expecting 40 particles is essentially zero if the test is performed correctly. The tracer test involves pouring a feed sample through a "Rotary Detector" laboratory magnetic separator isolating the tracer on a small filter paper, adding a solvent, (usually water) to dissolve the dye from the colored iron particles coloring the filter paper. A feed containing the coded coccidiostat will yield a bright ring of color on the filter paper, a feed supposed not to contain the premix should yield no color. Different colored tracers may be used to code different premixes. For a quantitative estimate of the tracer and by inference the medication, one "demagnetizes" the magnetic material and sprinkles it onto a large filter paper wetted with the appropriate developer, usually 50% ethanol, to yield a paper with countable spots.

The Microtracer test - practical considerations - potential problems

1) The sample must be representative of the truckload of feed. The feed should be a composite from various parts of the truckload. Even with great care in sampling, the potential for error is real when analysis of 500 grams of feed is used to characterize 20 metric tons.

2) The feed manufacturer must run a "control positive" each day, testing a feed known to contain the coccidiostat with tracer to be sure a feed positive for the medication yields a



positive result for the tracer. For qualitative testing, for most Microtracers, water must be used to develop the tracer color because other developers (i.e. pure ethanol) may yield little or no color. If the wrong solvent is used, a "false negative" result for the tracer will render the test meaningless.

3) The feed manufacturer must establish acceptance/rejection criteria. If one is expecting 40 colored spots from a test and finds 2, should the truckload of feed be considered contaminated and rejected? In such instances, it is probably best to take additional samples from each compartment on the truck and to analyze them. If no sample yields more than one or two spots and a "control positive" yields 30 or 40 spots or a bright ring of color as a qualitative test, then the trace contamination may be tolerable and the feed may be shipped. Each feed manufacturer must establish their own procedures and criteria as to what results are acceptable and what are not.

4) Interpretation of quantitative tracer counts is limited by the uniformity of the tracer, the consistency of tracer recovery at different feedmills and by the variability inherent to the applicable Poisson statistical distribution. The true count of the tracer may vary 10% between lots, the recovery of the tracer may vary 10% between feedmills and if one counts 100 tracer spots this count will have an inherent standard deviation of its square root or 10 and a resulting coefficient of variation of +/-10%. As a practical matter, if one counts 100 tracer spots from a feed sample, this count should allow an estimate of the level of the coded coccidiostat +/- 30-35% with 95% statistical confidence.

Actual data from three feedmills

Feedmill #1

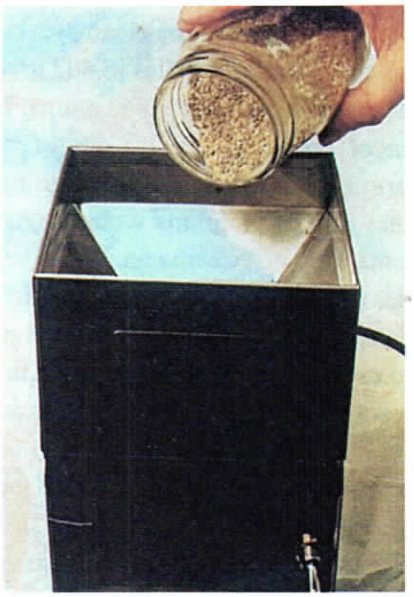
Microtracer F-Red with a specified count of 25,000 particles/gram was formulated in salinomycin to yield 3.6 grams of tracer per metric ton of feed when the formulated level of salinomycin was 60 grams/ton. This would yield a "theoretical" tracer count of 25.6 particles per 280 grams of feed if recovery of the tracer was 100%.

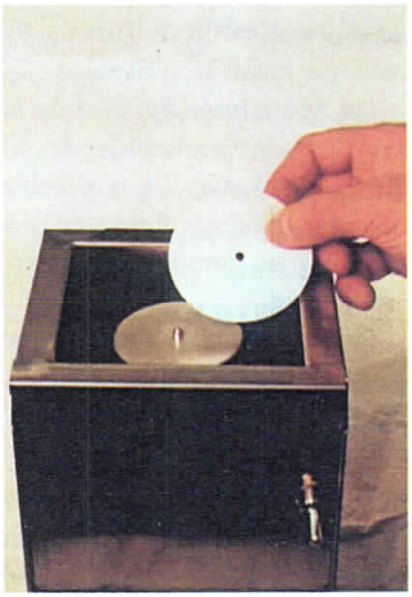
Samples were taken from 208 truck-loads of pelleted feed manufactured while the salinomycin/tracer premix was being used. Most but not all these samples were consecutive.

All these samples were analyzed for the tracer and twenty-three were analyzed chemically for salinomycin. The samples analyzed chemically were taken from each feed formula production run in the order these were produced. These formulas called for either 60 grams of salinomycin per ton of feed, 55 grams or none. The samples chosen were biased, however, with many chosen because the tracer result deviated from the feed manufacturers formulation. A total of 1,336 tracer particles were found in 63 samples formulated with salinomycin at 60 grams/ton. The recovery of the tracer was 82.8% of the formulated level. The recovery of the tracer from 50 feed samples taken from mash feed in the surge bin during this study was 95.7% of theory.

The apparent loss of tracer between the surge bin and the pelleted samples was 13.5%. A total of 583 tracer particles were found in 28 samples formulated with salinomycin at 55 grams/ton with an indicated tracer recovery of 89.3%.

A total of 98 tracer particles were found in 117 samples supposed to contain no salinomycin. This represented 4.8% of the total tracer found.





The results indicate that while the chemical assay results for the 23 samples analyzed were more precise in estimating the level of the medication in feeds, the tracer results were as efficacious in qualitatively identifying the drug. For sixteen samples, both the tracer and chemical assays correctly predicted the presence of the drug, for three samples the chemical assay corresponded with the drug formulation while the tracer did not and for four samples the tracer corresponded with the drug formulation while the chemical assay did not. In no case did both the tracer and chemical assay deviate from the formulated medication.

The level of detection for the chemical assays was 16 grams/ton or 25% the formulated level. Results were thus of little value in estimating contamination levels of the drug. Of the 91 samples formulated with salinomycin at either 60 or 55 grams/ton, 2 yielded tracer counts of 5 or less (1 and 4) compared with an average of 23.5 found in the other 89 samples. These two results may have been "false negative" results for the medication. Only the sample with a count of 4 was analyzed chemically, this yielding a positive chemical assay at 49.8 grams salinomycin/ton. Of the 117 samples supposed to contain no salinomycin, three yielded counts of 6 or more (6, 8 and 7) all 1/3rd or less tracer than found in the feeds formulated with the medication at 60 grams/ton. These three results would be considered "weak positives" for the medication. Only the sample with a count of 7 was analyzed chemically.

This yielded a positive chemical assay at 56 grams salinomycin/ton. The tracer may have correctly predicted a mistake. The salinomycin/tracer premix was formulated in approximately 60% of the tonnage being produced at the feedmill. Feeds supposed to contain no salinomycin on average contained tracer at 3.0% the formulated level.

Feedmill #2

Microtracer F-Special Blue was formulated in Nicarbazin premix to yield a theoretical count of 137 particles per kilo of final feed. Samples were taken from 115 truckloads of feed while the Nicarbazin/tracer premix was being used.

All these samples were analyzed for the Microtracer but none were analyzed by chemical assay for the medication. Two samples weighing a total of 863 grams were stated as containing Nicarbazin. One of these samples contained 41 tracer particles in 402 grams the other 38 particles in 462 grams. The tracer recovery was 75.4% of that formulated. 113 samples weighing a total of 52,821 grams were stated as containing no Nicarbazin. These samples together yielded 15 tracer particles. No sample contained more than 2 particles when results were adjusted to an average sample weight of 467 grams. Six of these samples weighing a total of 2,482 grams were of breeder feeds where sequencing and flushing procedures were employed to prevent Nicarbazin from reaching them. These samples contained no tracer particles.

Nicarbazin was formulated in approximately 10% of the feed manufactured during the study. Feeds supposed to contain no Nicarbazin on average contained tracer at 0.26% the level formulated. If Nicarbazin had been formulated in a greater proportion of the feed being manufactured, it is likely the level of tracer found in feeds supposed to contain none would have been higher than that found.

Feedmill #3

This feedmill was owned by the same poultry integrator as "Feedmill #2" but was of a different design.

Microtracer F-Special Blue was formulated as for Feedmill #2 and again 115 feed samples were analyzed for the Microtracer but not chemically for the medication.

Five samples stated as containing Nicarbazin all yielded counts of at least 35 particles from sample weights of between 435 and 535 grams.

A total of 201 particles were found in 2,498 grams of feed. The tracer recovery was 68.1 % of that formulated. 110 samples weighing a total of 53,952 grams were stated as containing no Nicarbazin. These samples together yielded 16 tracer particles representing 0.22% of the "theoretical" tracer expected in a feed formulated with Nicarbazin. No sample contained more than 3 particles.

Thirty of these samples weighing a total of 16,306 grams were of breeder feeds. These contained 10 tracer particles or 0.45% of the "theoretical" tracer expected in a feed formulated with Nicarbazin. Again, Nicarbazin was being formulated in approximately 10% of the feed being manufactured.

Conclusions

The Microtracer results at the two feedmills using Nicarbazin premix with tracer were excellent in qualitatively differentiating feeds formulated with the medication from those supposed to contain none.

The Microtracer results at the feedmill using salinomycin with tracer were not quite as good at differentiating feeds formulated with the medication from those supposed to contain none, though the tracer results were as good as chemical assays in doing so. Feeds supposed to contain no tracer yielded an average of 3.0% of the tracer expected from feeds formulated with salinomycin at 60 grams/ton.

The level of tracer contamination was higher than at the mills using Nicarbazin.

This may have been because the salinomycin was being formulated in a higher proportion of the feeds being manufactured.

Contamination of tracer into non-target feeds was successfully controlled at one mill where no tracer whatsoever from Nicarbazin was found in breeder feeds.

This was the most recently constructed feedmill and specific sequencing and flushing procedures were being rigorously employed.

Implications

Coccidiostats that may be toxic if present in non-target feeds at formulated levels or lower appear controllable.

With careful planning, tracer technology should make it possible for feedmills to use limited quantities of specific medications keeping contamination of such medications into non-target feeds at much less than 1% of the formulated level.

Feed additives that can cause illegal tissue residues in meat and poultry seem controllable at levels of concern. With further research, tracer techniques should make it possible to establish objective standards for acceptable contamination of medicated feeds into non-target feeds.

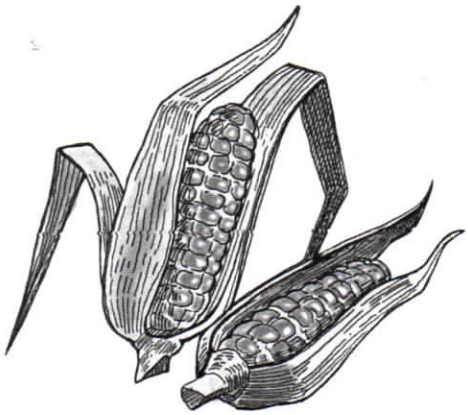
Chemical assays for salinomycin were costly, time consuming and not adequately sensitive to be of value for this purpose.

Bibliography

"Prevention of Anticoccidial Toxicity Using Microtracer Premix Additive", *Zootecnica International*, November 1993.

"The Use of Colored Iron Particles in Determining Coded Coccidiostats and Antibiotic Premixes in Finished Feeds", *AOAC International Forum: Methods for Antibiotic and Drugs in Feeds*, San Diego, California, September 1997.

The Use of Colored Iron Particles in Determining Cross Contamination of Medicated Feeds at Feedmills and Feed Premix Plants



Presentation of David Eisenberg of Micro-Tracers,Inc. (San Francisco) at AOAC Forum on *Methods for Analysis of Antibiotics and Drugs in Feeds* Los Angeles, 22 September 2002

NUTRITION

This topic is of current developing interest largely because the European Union and other major world markets are developing new food and feed regulations that are much more aggressive than what have existed in the past.

The European Union now requires that all feedmills whether they mix drugs in feeds or not must be registered with their national governments. Further, they must also have data validating the adequacy of their mixing and of their control of contamination at their feedmills and premix plants.

The issue of medicated feed contamination into non-medicated feeds is not new. In 1976, the US-FDA requested the Animal Health Institute (AHI) and the American Feed Industry Association (AFIA) (then American Feed Manufacturers Association) to submit recommended "Action Levels" that could be met by industry. These were to be the maximum levels of drugs in non-medicated feeds that would be considered acceptable or alternately the minimums that would lead to FDA enforcement actions. The AHI and AFIA did submit recommendations but opposed any action by the FDA on the matter and because of this opposition and possibly more so because the issues are complex, more than 25 years later no Action Levels exist and they probably will not exist for at least a few more years.

Why is it important to minimize cross-contamination of drugs into non-medicated feeds? Such residues at high levels (i.e. 20% of formulated levels) may be toxic such as Nicarbazin reaching breeder feeds, salinomycin reaching adult turkey feeds.or monensin reaching horse feeds. Such residues at trace levels into "finisher" feeds, the last feeds fed animals prior to slaughter, may lead to illegal residues of the drugs in the tissue of the treated animals, poultry or seafood. Past problems with sulfamethazine in pork and current problems with chloramphenicol would be examples. A related concern would be the issue of ruminant by-product reaching ruminant feeds where it could lead to transmission of BSE (mad cow disease).

1. Pouring a feed sample into a plastic weigh boat to obtain a given weight of subsample.

How is the industry currently testing to confirm manufacturing procedures are designed to minimize cross-contamination? Some manufacturers are testing for specific drugs formulated in their feeds, and using such results to establish procedures applicable to all drugs used at the feedmill.

Many drug assays are accurate and precise in detecting drug residues in meat and poultry as these are relatively simple matrices to work with. Formula feeds, however, often contain large numbers of ingredients that can make analysis for drugs at very low levels difficult or in some cases effectively impossible. It is possible to test for cross contamination of drugs into non-medicated feed when the drug is formulated at a very high level in the medicated feed or better premix and when it has a good assay at relatively low levels.

Another approach is to mix simple, easy to detect tracers most often colored iron particles into a medicated feed or premix and to determine the tracer rather than the drug, at least as a screening procedure. Currently, this approach is being actively investigated by TNO (Dutch Research Institute), Tecaliman (French Research Institute) and others, primarily in Europe.

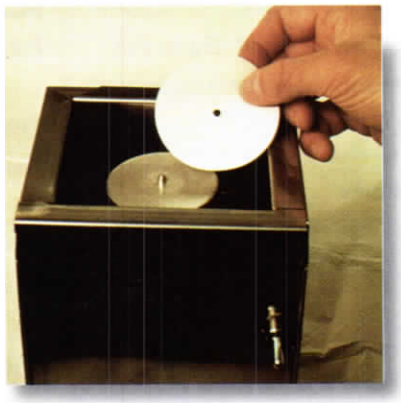
Micro-Tracers,Inc. (San Francisco) recently participated in such a Study as described below:

Study of Cross-Contamination of Coccicor Premix 2.5% Amprolium at Feedmill/Premix Plant:

Formulation of Tracers Into Medicated Premix:

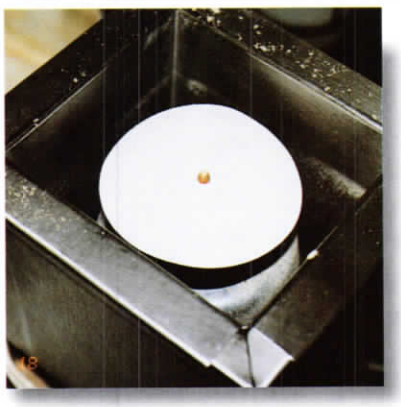
Slides 1 through 6 are applicable to both determining colored iron particulates- particle counts as well as determining color of very fine colored iron particles on a spectrophotometer.

2. The Microtracer "Rotary Detector".



3. Placing a 7.5cm filter paper with centerhole on the circular magnetic platform within the Rotary Detector housing.

4. Pouring the feed subsample through the upper hopper of the Rotary Detector.



5. Viewing on the 7.5cm filter paper within the Rotary Detector the magnetic materials removed from the feed subsample.

6. Brushing the magnetically retrieved material into a small aluminum weigh scoop, prior to determining particle counts or colorimetric readings.

Two tracers, red colored iron particles with a count of 25,000/gram (micron size 150-300) and fine blue "lake" colored iron powder (micron size 50-150) were formulated at 1-kilo each per 2,000-lbs. into one 2-ton batch of Coccicor 2.5% Amprolium Premix.

Sampling: Medicated Premix and Three Following Batches of Premix (pelleted) Supposed to Contain No Drug (or tracer).

TABLE I ***Tracer Results***

Sample	*Weight Analyzed*	*Red Count-Color Absorbance**		*Blue Color Absorbance**
Coccicor Mixer Sample-	4 gms.	121	0.280	0.415
Coccicor-Average of Samples from Mixer, Conveyer, Elevator, Cooler Pallets and Packer Pellets-	4 gms	100	0.229	0.305
Batch #1 Following-				
Mixer-Avg. five samples-	200 gms	21	0.062	0.091
Mixer Sample #1-	"	20	0.075	0.110
Conveyer-Avg. five samples-	200 gms	395	0.59	0.675
Conveyer Sample #1-	"	985	2.67**	1.8**
Elevator-Avg. five samples-	200 gms	144	0.381	0.380
Elevator Sample #1-	"	173	0.468	0.450
Batch #2 Following-				
Mixer-Avg. five samples-	200 gms	0.4	Nil	Nil
Mixer Sample #1-	"	1	Nil	0.005
Conveyer-Avg. five samples-	200 gms	47.4	0.105	0.143
Conveyer Sample #1-	"	104	0.212	0.255
Elevator-Avg. five samples-	200 gms	37	0.083	0.081
Elevator Sample #1-	"	24	0.090	0.120
Batch #3 Following-				
Mixer-Avg. five samples-	200 gms	1.2	Nil	0.008
Mixer Sample #1-	"	0	Nil	Nil
Conveyer-Avg. five samples-	200 gms	19.4	0.037	0.058
Conveyer Sample #1-	"	59	0.122	0.220
Elevator-Avg. five samples-	200 gms	5.8	Nil	0.009
Elevator Sample #1-	"	7	Nil	0.008
Batches #1, 2 and 3 combined(Pellets)				
Cooler-Avg five samples-	200 gms	36.6	0.065	0.045
Cooler Sample #1-	"	35	0.060	0.031
Packer-Avg. five samples-	200 gms	50.8	0.059	0.045
Packer Sample #1-	"	105	0.100	0.058

* tracers retrieved from premix samples using Micro-Tracers,Inc. Rotary Detector laboratory magnetic separator. For color readings, diluted to 15 ml in 1% sodium carbonate solution and read at 525nm and at 630 nm, the wavelength maxima for FD&C Red#3 (erythrosine) and FD&C Blue#1 (Brilliant Blue FCF).

** for this sample only, for color reading tracer was diluted in 100 ml of 1% sodium carbonate solution with results calculated and reported to a 15 ml dilution basis.

From Coccicor 2.5% Amprolium Premix-

One sample at each of five locations: #1. Mixer, #2. Conveyer (after Discharge of Mixer), #3. Elevator (at top of feedmill above pelletmill), #4. Cooler (after pelleting) and #5. Packer (pellets at truck loading).

From each of the following three Batches of Premix-

Five samples from each of three locations: #1. Mixer, #2. Conveyer and #3. Elevator.

The three Batches of Premix were then comingled and additional samples were taken-

Five samples from each of the following: #4. Cooler and #5. Packer.

A total of 60 samples were taken, five of the Coccicor 2.5% Amprolium Premix and fifty-five of following premixes not formulated with the drug.

Analysis of Samples:

All samples were analyzed for the red colored iron particles and for the fine blue "lake" iron powder. The tracers were isolated from the premix by magnetic separation using a "Rotary Detector" (1) equipped with a special "rare earth" magnet.

After isolating the tracers from the premix, the red tracer was demagnetized, sprinkled onto a large filter paper, sprayed with a mist of 50% ethanol with 0.5% ammonium hydroxide, the paper dried and the resulting red colored spots counted. The blue color was diffuse, not countable.

A second subsample was also analyzed, isolat-

ing the two tracers from the premix via magnetic separation. Instead of developing colored spots on a filter paper, the magnetic material was brushed into a centrifuge tube, diluted with 1% sodium carbonate solution, shaken on an "auto" shaker for ten minutes, and the two colors read on a spectrophotometer.

To maximize sensitivity of the tracer methods, four gram samples of the Coccicor 2.5% Amprolium Premix were analyzed while 200 grams of all non-medicated samples were analyzed.

unless otherwise noted) into following premix production was as follows:

		Red Particle Count-	*Color*	*Blue Color*
Batch #1-	Mixer	0.38%*	0.40%*	0.48%*
	Conveyer	7.2%	3.8%	3.6%
	Conveyer Sample #1	17.9%	17.2%	9.5%
	Elevator	2.6%	2.5%	2.0%
Batch #2-	Mixer	0.01%	Nil	Nil
	Conveyer	0.86%	0.68%	0.75%
	Conveyer Sample #1	1.89%	1.37%	1.34%
	Elevator	0.67%	0.53%	0.43%
Batch #3-	Mixer	0.02%	Nil	Nil
	Conveyer	0.35%	0.24%	0.30%
	Elevator	0.11%	Nil	Nil
Sample Batches #1, 2 and 3 Combined (pellets)-				
	Cooler	0.67%	0.42%	0.24%
	Cooler Sample #1	0.64%	0.38%	0.16%
	Packer	0.92%	0.38%	0.24%
	Packer Sample #1	1.90%	0.65%	0.30%

*All values are percentage of originally formulated tracer not adjusted for recovery.

Eight samples were analyzed chemically for Amprolium. These samples were chosen only after all tracer analyses had been completed. Samples were not chosen for chemical analysis at random but rather because they were thought interesting (**Table 1).**

The five samples taken from the Coccicor 2.5% Amprolium Premix yielded tracer "recoveries" as follows when compared to results for analysis of the pure tracers:

	Red Particle	Count- Color	Blu Color
Mixer-	110%	90%	109%
Conveyer-	93%	68%	84%
Elevator (after)-	77%	45%	65%
Cooler (pellets)-	89%	90%	69%
Packer (pellets)-	84%	76%	73%

The Coccicor-Mixer sample was analyzed chemically with a result of 2.02% or a recovery of 80% of the specified drug.

The Coccicor Packer sample was analyzed with a result of 1.79% or a recovery of 72%.

Cross Contamination of the Coccicor 2.5% Amrpolium premix (average for 5 samples

The chemical assay data generated to date is very limited. The chemical assay data and the tracer estimates for these samples are as follows:

Sample	Chemical Assay *ppm*	Red Tracer-Count-Color *ppm*		Blue Tracer-Color *ppm*
Coccicor-				
Mixer Sample- (25,000ppm)	20,200	27,500	22,500	27,300
Coccicor- Packer Pellet-	17,900	20,900	22,500	17,000
Batch #1-				
Mixer #1 Sample-	370	90	120	140
Conveyer #1 Sample-	4,170	4,470	4,380	2,360
Elevator #1 Sample-	730	790	760	590
Batch #2-				
Mixer #1 Sample-	150	Nil	Nil	Nil
Conveyer #1 Sample-	660	470	340	340
Conveyer #2 Sample-	170	130	160	290

Sample Calculations:

1. Red Colored Iron Particles-

Particle Counts-

Tracer specification (and estimated count): 25,000/gram

Formulated at 1-kilo per 2,000-lbs. of Coccicor 2.5% Amprolium premix (all 2-ton batches)

Estimated particles added to premix per 2,000-lbs: 25,000 X 1,000 grams = 25,000,000.

Estimated particles per four (4) gram subsample of premix: 25,000,000 divided by 2,000-

lbs. divided by 454 grams X Four (4) grams = 110 particles

Actual count from Coccicor premix sample taken from Mixer: 121 (from 4 grams)

Estimated Tracer Recovery: 110% of formulated

Estimated Amprolium: 110% X 25,000pp, specification of premix = 27,500 ppm (tracer estimate could be made more accurate by analyzing a larger sample and counting more particles).

2. Particulate Red Iron Particles- Color Readings-

Absorbance found from 0.0044 grams of tracer (the amount formulated per 4 grams of Coccicor premix) diluted in 15ml of 1% sodium carbonate in water solution = 00.309

Absorbance found from tracer from 4 grams of Coccicor premix- Mixer sample = 0.280.

Estimated Tracer Recovery: 90.3%

Estimated Amprolium: 90.3% X 25,000 ppm specification = 22,500 ppm

Absorbance found from tracer recovered from 200 grams of followup Batch #1 Conveyer #1 sample, tracer diluted in 100 ml of 7% sodium carbonate in water solution = 0.400, adjusted to dilution in 15ml = 0.400 X 6.67 = 2.70 divided by 4/200 = 0.540

Estimated Tracer Recovery = 17.5%

Estimated Amprolium: 25,000 pp, X 17.5% = 4,380 ppm

Linear regressions of this limited data yielded equations and correlation coefficients as follows:

Chemical amprolium assays with red particle counts: Y = 332.7 + 0.767 X, correlation coefficient 0.996

Chemical amprolium assays with red color readings: Y = 229.4 + 0.838 X, correlation coefficient 0.997

Chemical amprolium assays with blue color readings: Y = 754.5 + 0.798 X, correlation coefficient 0.978

7. Brushing magnetic material into test tube with given volume of solvent to dissolve dye from very fine iron particles.

8. Shaking test tube to dissolve dye from tracer particles.

9. Reading color of solution on spectrophotometer.

10. "Demagnetizing" the magnetic materials with bulk tape eraser so they do not stick together after magnetic separation from the feed (required for determining particle counts).

11. Spraying large 18.5 cm filter paper with solvent prior to determining tracer counts.

12. Dried 18.5cm filter paper with red particle spots and background fine blue but uncountable color from the very fine colored particles.

Conclusions:

1. The three tracer procedures- red particle counts, red and blue color readings yielded data that reflected the presence of the coded drug adequately to at least be used to screen samples for the much more expensive chemical analysis.
2. It appeared that through sequencing (no flushes were employed) cross contamination of the drug into non-medicated following feed was kept at 1% or less based on the average tracer results of final product at loadout. This low result was achieved, however, by blending three batches of following product together. If the immediately following batch had been kept isolated through the mill, cross contamination into the packed product would have probably been between 2.% and 3.5%.
3. In testing for cross contamination at feedmills and premix plants it is critical one know and understand the flow of materials through the plant. This will allow generating meaningful information as to where and when contamination is occurring so that properly engineered solutions may be tried.
4. It is also important to know the physical properties of the medicated premix being studied, as powdered products most likely contaminate more than granulated ones though this was not evidenced by the data generated in this Study.
5. Contamination is usually concentrated in the first sample taken from following production. It also would increase as product flows through a manufacturing plant, though in this case the highest average levels of contamination occurred from samples taken at a conveyer exiting the surge bin.
6. Surprisingly, the red particulate tracer yielded decent colorimetric readings even from pelleted feed.
7. Interpretation of the data requires consistency. Should cross contamination results be compared with the specified level of the drug or with the amount of drug found in the formulated batch based on chemical assay of it.
8. Approximately 20 hours of laboratory time was required to generate the one-hundred and eighty tracer results generated and the value of the tracers consumed was less than $100. The cost of validating cross contamination procedures at feedmills would seem trivial though the engineering costs involved in solving problems found could be very great.