GENERAL MANAGEMENT CONSIDERATIONS

Coccivac[®] Live Coccidial Vaccines

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GENERAL MANAGEMENT CONSIDERATIONS Coccivac[®] Live Coccidial Vaccines

Coccivac[®] vaccines are a suspension of live sporulated coccidial oocysts representing the pathogenic *Eimeria* species that commonly infect chickens. These vaccines are designed to infect the bird with a controlled number of organisms to stimulate a protective immune response. For this immune response to be effective, live coccidial vaccination programs depend on quality, healthy chicks in good environmental conditions.

While chickens are susceptible to coccidial infection at any age, exposure typically occurs in the first two weeks after placement. Following initial controlled exposure, coccidia must achieve two or more subsequent life cycles in the bird for full immunity to develop, depending on the *Eimeria* species. Protective immunity is often achieved at approximately three weeks of age with no obvious signs of clinical disease, and with repeated low-level exposure to coccidia, this immunity will last throughout the life of the chicken.

Immunity to *Eimeria* is species-specific, meaning one *Eimeria* species will not provide protection against another *Eimeria* species. Although the initial dose and route of administration are controlled, subsequent cycling of the vaccine is heavily influenced by poultry house management conditions as well as the general health of birds and their immune status.

This document is intended to highlight general management considerations for broiler chickens vaccinated with Coccivac[®]-B52, although many sections will also have relevance to birds vaccinated with Coccivac[®]-D2.

Coccivac vaccines

Each serial of Coccivac is evaluated microscopically and carefully titrated to ensure serial-to-serial consistency for uniform field performance. Vaccine safety, quality and consistency are further backed up by live bird testing of each serial prior to release for sale.



Coccivac®-B52

Coccivac-B52 contains 5 anticoccidial-sensitive strains of *Eimeria* (*Eimeria acervulina*, *E. maxima*, *E. maxima* MFP, *E. mivati* and *E. tenella*). It is intended for use in broiler (meat-type) chickens.



Coccivac[®]-D2

Coccivac-D2 contains live coccidial oocysts prepared from 6 anticoccidial-sensitive strains: *Eimeria acervulina, E. maxima, E. mivati, E. tenella, E. brunetti* and *E. necatrix*, to aid in the prevention of coccidiosis in chickens. It is intended for use in broiler breeder replacement pullets and egg laying pullets.

VACCINATION



Vaccine Storage and Handling

Coccivac vaccines are formulated to provide an adequate initial dose of live sporulated oocysts to the bird. Sporulated oocysts are sensitive to adverse environmental conditions, especially temperature.

- Carefully read and follow label instructions for storage, mixing and administration of live coccidial vaccines to prevent unnecessary vaccine titer loss.
- Coccivac vaccines should be maintained at a temperature between 35°F to 45°F (2°C and 7°C) throughout shipping and storage.
- Avoid freezing Coccivac vaccines! Freezing will kill or damage sporulated oocysts, resulting in a less effective vaccine. If a vaccine shows evidence of accidental freezing by visual presence of ice crystals or refrigerator monitoring detects low temperatures, the vaccine should not be used.

Vaccine Administration

Uniform Vaccine Administration and Effective Oocyst Ingestion are Essential

- Chick quality and Coccivac success are intrinsically related. It is imperative to have good quality chicks along with uniform application.
- Uniform vaccine coverage and ingestion of the vaccine at the hatchery ensures all birds begin the cycling process together. This will encourage uniformity of subsequent coccidial cycling and development of immunity in the field.
- Improperly vaccinated chicks at the hatchery, due to poor spray application, improper mixing or malfunctioning equipment, etc. may be naïve at placement. These chicks will receive their initial exposure to coccidia from field strains or higher first cycle vaccinal oocyst output from flockmates. These potential uncontrolled dose exposures can be overwhelming and may result in necrotic enteritis issues early in the life of the flock.
- Coccivac vaccines are approved for hatchery administration via the Spraycox[®] application system. Uniform vaccination is best achieved using the Spraycox approved hatchery application system.

Vaccine Application Methods

Hatchery Spray

Spraycox[®] II and Spraycox X[™] spray cabinets have been developed to accommodate hatchery design and chick processing volumes. Only these application systems should be used for Coccivac vaccines.



Latest generation Spraycox X spray cabinet for Coccivac application in the hatchery.

The Spraycox cabinet delivers a uniform 21 ml dose by coarse spray for each box of 100 chicks. Monitor equipment daily to ensure proper calibration of volume and spray pattern.



Example of evenly covered chicks following vaccination with Coccivac when using red dye.

- Initially after vaccination, chicks will be slightly damp with an even coloring of red dye signifying good spray application.
- Chicks will begin to clean, dry and oil their feathers after vaccination (an instinctive behavior called "preening"). Preening should be encouraged, since this is the method by which oocysts are ingested.
 - Hatchery holding rooms should be well lit and maintained at temperatures, which support chick comfort and encourage preening behavior, not human comfort.
 - Allow at least 15 minutes for chicks to preen and feathers to dry in the warm environment of the hatchery.
 - -The red dye added to Coccivac helps stimulate the preening behavior and monitor uniform coverage.
 - Use only Merck approved red dye, since some dyes or coloring agents may be harmful to the sporulated oocysts in Coccivac.
 - Recommended dye concentration is 0.2 mL of Merck red dye per 1,000 doses. (2 mL per 2100 mL of diluted vaccine.

Hatchery Gel

For successful coccidial vaccination, oocysts must reach the chicks in a uniform manner. This has been shown to be accomplished regardless of vaccine or diluent used. No differences in body weight gain or protection were observed from challenge between birds vaccinated using coarse spray, highly viscous gel, low viscous gel or via gavage with Coccivac-B52.^{1,2}

Gel considerations

- Carrying capacity of a gel droplet is dependent on size of the droplet
 - Large droplets contain more oocysts than smaller droplets
 - Type of coccidial vaccine given by gel influences the number of oocysts per milliliter
 - Proper gel application is highly dependent on uniform mixing, especially if respiratory vaccines are also included.

When either method is used properly and chickens are exposed to an appropriate dosage of coccidial vaccine, protection will be achieved, regardless of vaccine application.

POULTRY HOUSE MANAGEMENT

Brooding Considerations

Placement Density

Initial stocking density plays a critical role in subsequent cycling of Coccivac oocysts in the chicken house. Higher bird concentration (high density) may result in litter moisture control issues and result in higher exposure to more infective oocysts. Lower bird concentration (low density) may result in less sporulation and less exposure to infective oocysts. Release of birds from the brood chamber must be managed properly, in order to promote continuous recycling of oocysts and consequent development of immunity. As a result, high density flocks may require release from partial-house brooding earlier than low density flocks, even during winter months. When considering stocking density, feeder and drinker space should not be sacrificed.



Coccivac vaccinated chicks in a broiler house.

Bedding

Poultry house litter management is extremely critical to the success of a coccidiosis vaccination program and broiler production in general. Depending on the region, several options for litter material exist. Pine shavings are the most common product available to poultry growers and have proven most effective with moisture management. Avoid litter material derived from hardwoods, as *Aspergillus fumigatus* often contaminates these products.

Regardless of the material utilized or coccidiosis control program employed, good quality, built-up litter affords growers the best means of managing moisture and heat within the poultry house environment. The ability of built litter to retain moisture, maintain beneficial bacterial flora and generate heat, complements modern broiler production. A good litter base of 4 to 6 inches is preferred. Litter moisture is an indication of the relative humidity (RH) in the chicken house. A certain level of RH is needed for fecal droppings to remain moist long enough to initiate the sporulation process. Good sporulation can be achieved when the moisture content of the litter is 25%. At this level of moisture content, a handful of litter can be formed into a ball, which will temporarily hold its shape before falling apart. If the litter remains in a ball, this suggests the moisture content may be 35% or higher, which is excessive. Maintain good litter conditions in the house by avoiding extreme conditions of too dusty or wet/caked litter.

Brooding Temperature

Air and litter temperatures help maintain a thermoneutral zone for chicks.

- Comfortable chicks start eating and drinking faster. Air and floor temperatures should encourage good activity and not impair feed and water consumption.
- Chick behavior is directly correlated to rectal temperatures. A chick's rectal temperature should be maintained between 103.2° F to 104.5° F (39.5° C to 40.3° C).

Feed Management

Supplemental feeders ensure young birds get a good start in their new environment by making feed readily accessible.

- When partial-house brooding, supplemental feeders (feeder lids) should not be reduced or discontinued until birds are at least 10 days of age and ready for transition to full house. Continue supplemental feeder use in the full house until birds are fully acclimated to the standard house feeders. This may be accomplished by moving supplemental feeders from the brood area to the unused section(s) of the chicken house to encourage timely migration after turnout.
- Supplemental feed should always be removed gradually to avoid mortality spikes or litter eating. This becomes even more critical when birds are large enough to occupy space in feeder pans further limiting access to feed.

- If feed access is limited, chicks may forage more in the litter. Consumption of excessive amounts of litter material is not nutritionally sound and may contain potentially high levels of bacteria such as *Clostridium spp*. Litter eating, in a subpopulation of birds, can potentially lead to necrotic enteritis along with reduced flock uniformity and performance.
- Avoid making feed changes during the peak coccidial vaccine cycling (every 7 days). For example, transitioning from starter feed to grower feed at 14 days of age.
- Monitor crop fill 24 and 48 hours after placement.
 - Full crop targets:
 - 95% at 24 hours
 - 100% at 48 hours

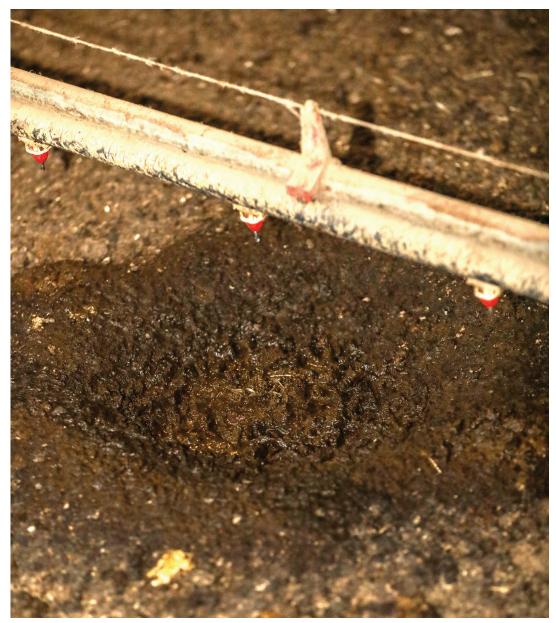


Example of readily accessible feed during brooding.

Water Management

In chickens, feed consumption is highly correlated with water consumption. Feed consumption decreases with limited water access and vice versa.

- Supplemental waterers are typically used with replacement pullets and less commonly in broilers. They should be used until birds are fully acclimated to the standard house water system.
- Properly manage height and water pressure of nipple drinker systems to avoid excessive moisture under the water lines.
- Proper water line sanitation is imperative to keeping birds healthy throughout the grow out period.



Example of wet litter associated with leaking nipple drinker.



Proper drinker height is important for maintaining dry litter conditions.

Litter Management

Although adequate litter moisture (~ 25%) is necessary for sporulation and completion of the coccidial life cycle, excess moisture may negatively impact Coccivac. Excessive litter moisture can increase sporulation potential, resulting in higher coccidia exposure for the flock during the second and third in-house coccidial cycles. Additionally, litter moisture can lead to increased amounts of bacteria that can place a more significant burden on the chicken's immune system, especially *Clostridium spp.*, which can lead to necrotic enteritis, while *Escherichia coli* has been associated with inflammatory process (IP), and airsacculitis. Additionally, excessive litter moisture is associated with high ammonia levels resulting in; burned footpads, corneal ulcerations, immunosuppression, chilled birds, poor flock uniformity and is considered an animal welfare issue.

- Maintain proper ventilation and water management to keep litter in good condition.
- Use proper litter depth of absorbent, high-quality litter material during the brooding period.
- Prepare litter prior to chick placement.
 - Remove caked surfaces
 - Level litter to ensure proper drinker height throughout the house
 - Use litter amendments to decrease pH and reduce ammonia levels
 - Windrowing of litter has proven effective in lowering bacterial pathogens and coccidial oocysts before the next flock, when a minimum internal temperature of 130° F (54.4° C) is reached

Partial vs Full-House Brooding

Partial-house brooding can be an effective management tool to achieve the classic Coccivac cycling patterns of successful operations. This management practice is critical for younger aged broilers with shorter out times between flocks. The full-house brooding management practice can be successful, especially in warmer seasons, but should be limited to larger size bird programs or small bird programs that have adequate downtime between flocks

- Low-density flocks may require adjustments to brooding space to increase coccidial vaccine exposure in the birds during the second and third in-house coccidia cycles.
- High-density flocks may require increasing brooding space earlier than 14 days, even during cooler seasons, if litter moisture becomes excessive and feeder space becomes limited.
- A controlled release or full-house brooding program minimizes the stress of turnout.
 - Birds have less distance to travel once released
 - Controlled release ensures uniform vaccine re-exposure for proper re-cycling of oocysts
 - Controlled release should be managed based on environment and/or management (litter) conditions
 - Full-house brooding ensures even distribution of birds
- When placing birds in full house, migration fences can be set up to allow for equal distribution of birds within pens
 - Full-house brooding is only recommended on reused litter.

Lighting Management

Avoid highly restrictive lighting programs, which can result in accidental feed restrictions.

- Bright lights in the brood area encourage good chick activity and attract birds to the feed. A minimum light intensity of 3-foot candles (32.3 lux; ideally 4-foot candles or 43.1 lux or greater) should be used during the first week of life. Use crop fill targets to determine if lighting in the brood area is adequate.
- Improper lighting programs can lead to litter eating.
- Be aware, animal welfare programs may inadvertently constrain some lighting programs.

Ration Formulation

Ration formulation can be designed to maximize the growth pattern expected from Coccivac vaccinates as well as minimize any secondary bacterial challenges, which may induce enteritis.

- Proper rations should consider the early protein requirements of vaccinates, especially sulfur amino acids levels. Poultry industry data analysis suggests improved levels of total sulfur amino acids during the first 2 to 3 weeks of life may be linked to better performance in broilers receiving Coccivac-B52 vaccine.
- Pay attention to the digestibility of raw materials in starter feeds to preserve gut integrity.
- Extra fortification with fat-soluble vitamins (D and E) during peak vaccinal oocyst cycling may improve performance. Vitamin E supplementation at 40,000 to 60,000 IU in the starter feed is recommended.
- Avoid anticoccidial drug use during the first 2 weeks after Coccivac vaccination as they can disrupt normal cycling.
- Avoid extremely low protein diets from day 28 through slaughter, which may impact the compensatory gain effect.
- Addition of feed additives with anti-clostridial activity may be considered to help reduce incidence of necrotic enteritis during immunity-building phase of vaccination.
- Wheat, bakery meal, DDG's, barley or rye can play a significant role in intestinal irritation causing necrotic enteritis. Incorporate diet appropriate enzymes to help improve ingredient digestibility and minimize the incidence of necrotic enteritis.
- Dietary calcium, phosphorus and phytase may impact the natural necrotic enteritis cyclic pathway. Special attention should be paid to calcium source, type, size and level of inclusions within the diet. Research results suggest that dietary Ca level may influence NE-associated mortality.³

Feed Milling and Delivery

- Good communication between live production and feed mill personnel is essential when starting Coccivac to ensure non-medicated starter feed is properly delivered to the correct farms. Advance feed delivery may preclude last minute placement changes.
- Avoid mixing feeds containing anticoccidials and unmedicated feeds in storage bins, delivery vehicles and on the farm.
- Limited finish feed storage at some mills may require delivery of medicated starter feed to farms before beginning production of unmedicated starter feed.
- Flocks receiving an accidental feed delivery containing anticoccidials, during the 3-week period following Coccivac vaccination should be carefully observed as the potential for a coccidiosis break can occur. This may warrant keeping the flock on an anticoccidial ration for the duration of the growout period.
 - Special attention by producers, to feed deliveries during the transition period, may help preclude any corrective action later.



Multiple feed bins improve on farm feed management.

MONITORING

Crop Fills

Despite providing adequate feed and water, some chicks do not eat or drink after placement. Without proper monitoring producers may be unaware of these 'non-starter' chicks until it is too late and seven-day mortality spikes. An important step for starting well on Coccivac, is to ensure chicks begin eating and drinking soon after placement. This requires early monitoring of the chicks eating and drinking habits post-placement.

- Check crop fill at 24 and 48 hours after placement.
- Randomly select 100 chicks and palpate crops.
 - Full, soft and rounded crop = chick has found feed and water
 - Full, but hard crop = chick has found feed, but not water
 - If crop is empty, chick has not found feed or water
- Crop fill targets: 95% at 24 hours and 100% at 48 hours post-placement.
 - If crop fills are below target levels:
 - increase number of supplemental feeders
 - increase bird activity by walking the house
 - increase light intensity
 - modify house temperature to improve chick comfort level

Rectal Temperatures

Monitoring rectal temperatures is a good method to assess proper brooding. Additionally, evaluating bird behavior can be helpful in determining if brooding temperatures are adequate. Huddling suggests chicks are cold. Loud screaming chicks avoiding supplemental heat are most likely hot.

- Use a medical ear thermometer to monitor chick rectal/vent temperature, within the first 48 hours of placement. Monitor healthy chicks by gently pushing up the tail to expose the vent and placing thermometer tip on bare skin of vent area.
- Rectal/vent temperature target should range between 103.2° F to 104.5° F (39.5° C to 40.3° C).

Litter Moisture

Litter moisture plays a key role in uniform Coccivac cycling and is an indication of the house relative humidity (RH). A minimum of 25% litter moisture is needed for fecal droppings to remain moist long enough to achieve adequate sporulation. Avoid litter moisture extremes of dry and dusty or wet and caked.

Monitor litter moisture several times during the brooding period. Forming a ball with a handful of litter, which temporarily holds its shape before falling apart is a quick and easy method to determine adequate litter moisture (RH). If the litter remains in a ball, then moisture content is \geq 35%, which may result in excessive sporulation and higher oocyst levels.



Adequate litter moisture is important for good coccidial cycling. Avoid too much litter moisture (sample on the right, above), which can cause higher oocyst levels.

Coccivac in the Field

A successful Coccivac vaccination program depends initially on proper vaccine handling and uniform vaccine application at the hatchery. In the chicken house, uniform Coccivac cycling will result in development of an immune response effective in protecting against the major species of *Eimeria* responsible for coccidiosis in broilers with minimal to no post-vaccination reaction.

	E. acervulina	E. maxima	E. tenella
Peak Lesions	18-21 days	21-26 days	20-23 days
Gross lesion +1	Up to 15% @ 20 d	0%	≤5%
Gross lesson +2	≤10% @ 20 d	0%	0%
Microscopic lesion +1 (1-10 oocyst/100x field)		30-50%	
Microscopic +2 (11-20 oocyst/100x field)		≤10-20%	
Resolved	22 days	27 days	24 days (cecal cores rare)

Ideal Coccivac Lesions After Vaccination with Coccivac

A postmortem examination of vaccinated birds of varying ages, provides the best method to monitor Coccivac and evaluate various coccidial cycling patterns and confirmation of expected immunity development. Documentation should include gross lesions of *E. acervulina*, *E. maxima*, *E. tenella* and *E. mivati* along with microscopic oocyst enumeration of *E. maxima* to ensure each coccidial species fall within the expected lesion guidelines for Coccivac. This is also a good opportunity to evaluate overall bird health. A postmortem examination should be done prior to starting a Coccivac program in order to understand the current coccidiosis program's burden and potential carryover.

- Once a Coccivac program has begun, a health survey can be performed when the first vaccinated birds reach 28 days of age. Select birds in the 14 to 28-day range for evaluation of gross lesions and microscopic evidence of *E. maxima* cycling. Minimally, at least two flocks of the same age should be evaluated over the following age ranges:
 - <17 days
 - 18 to 21 days
 - 22 to 24 days
 - 25 to 28 days

- When a large enough sampling of birds is examined, a good picture of coccidial cycling patterns can be observed.
- If the health survey shows irregularity in the cycling pattern, field visits to several farms may be needed to look for signs of any abnormality or management practices not compatible with controlled vaccine cycling.
- Subsequent, routine posting sessions should consist of vaccinated birds from 14 through 35 days (or longer for big birds) using the sampling guidelines of 5 birds per flock, with 2-3 flocks per interval age range to ensure a good evaluation of the Coccivac program for expected cycling and immunity development. Minimally, two flocks for each age range should be evaluated over the following age ranges:
 - <17 days
 - 18 to 21 days
 - 22 to 24 days
 - 25 to 28 days
 - 29 to 32 days
 - 33 to 36 days
 - 37 to 41 days
 - 42 to 48 days
 - 49 to Market age

Coccidiosis Lesion Index

Coccidiosis Lesion Index (CLI) is an indicator of the average coccidial score of all the birds examined for each species of coccidia. A higher CLI indicates more coccdia cycling was seen in the birds. The CLI is calculated by multiplying the numerical lesion scores (1-4) by the number of affected birds then dividing by total number of birds examined. The CLI may be a helpful value for evaluating Coccivac performance over an extended time period.

An example of the CLI calculation for <i>E. maxima</i>
Total of 60 birds examined for <i>E. maxima</i> (microscopically)
Total of 16 birds with <i>E. maxima</i>
5 birds with Score 1: 5 birds X 1 = 5
6 birds with Score 2: 6 birds X 2 = 12
3 birds with Score 3: 3 birds X 3 = 9
2 birds with Score 4: 2 birds X 4 = 8
Total composite lesion score = 5 + 12 + 9 + 8 = 34
34/60 = 0.567 <i>E. maxima</i> microscopic CLI

Anticoccidial Sensitivity Testing (AST)

Anticoccidial Sensitivity Testing (AST) is a method used to determine the level of resistance or loss of sensitivity to in-feed anticoccidials. An AST can be useful to demonstrate loss of efficacy by in-feed anticoccidials and whether an alternative control method such as Coccivac may be warranted to improve performance and restore sensitivity to the coccidial population in the chicken house.

First, oocysts are isolated and propagated from fresh, randomly collected fecal samples. Test groups are based on in-feed products to be tested along with a positive and negative control. Microscopically identified sporulated oocysts are quantified for inoculation into chickens at a specified age (10-14 days) at doses high enough to cause disease. Inoculated chickens are evaluated after 6 days based on lesion scoring, bodyweight gain and feed conversion with treated groups compared against inoculated controls. The information obtained from the AST can help determine, which anticoccidials are more effective against the different species of *Eimeria*. An AST can be used to evaluate in-feed products used in conjunction with Coccivac, especially in antibiotic-free production.

For more information on performing an AST, please consult Merck Technical Service.

Oocysts Per Gram of Feces (OPG)

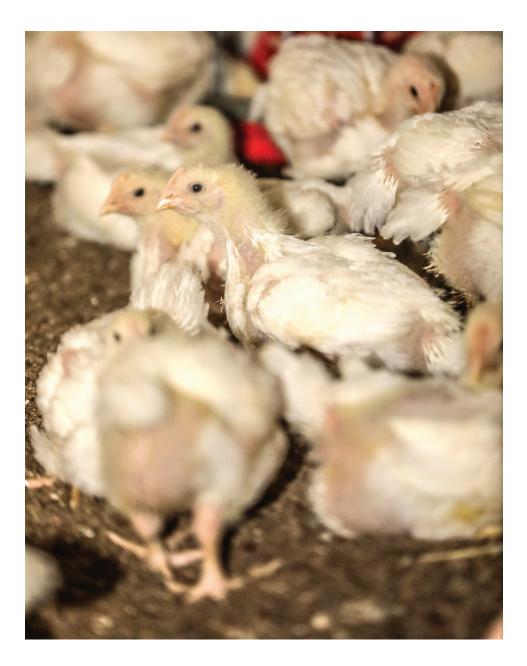
The oocyst per gram (OPG) technique is another useful tool to monitor resistance development to in-feed anticoccidials or onset of immunity. For proper monitoring, fresh fecal and cecal samples are randomly collected throughout the chicken house regularly (e.g. every three days) to generate a coccidial oocyst shedding curve. This tool can be used to determine when coccidial challenge is greatest, peak oocyst count levels, and field management can have an impact on peak oocyst timing and height.

For more information on setting up an OPG monitoring program, please consult Merck Technical Services.

TROUBLESHOOTING GUIDE

If monitoring programs yield a coccidial lesion profile which does not fall within expected Coccivac guidelines, or there is necrotic enteritis, use troubleshooting checklist below.

- Frozen or partially frozen vaccine: Check refrigerator for uneven internal temperatures, which may result in some frozen vials. If Coccivac is suspected to have been frozen, the vaccine should not be used. Contact your Merck Animal Health representative, so vaccine can be evaluated microscopically for evidence of damage consistent with freezing.
- Was proper vaccine dose administered?
 - Check volume of water (21 ml) applied to chick box?
 - Was correct amount of Merck red dye used?
- Monitor vaccine spray pattern in chick box for uniformity.
- Were chicks provided adequate lighting, temperature and time to preen at the hatchery?
- Was there a carryover effect from previous coccidial control program? If the previous in-feed anticoccidial program prior to Coccivac was losing efficacy, large number of viable oocysts can remain in the litter for possible early exposure by the next flock of chicks.
- Check starter feed for accidental inclusion of anticoccidial medication.
- Ensure bird density is not too high or too low to negatively impact vaccine cycling?
- Is litter management acceptable? Minimum ventilation, condensation associated with cold weather, heavy bird concentration and incorrect ration formulation may result in litter with high moisture content or caking.
 - Is litter too dry to support adequate oocyst sporulation?
 - Is litter too wet, allowing excessive sporulation?
 - Is litter depth adequate?
 - Is litter material absorptive?
 - Do birds have consistent exposure to infective oocysts for proper in-house vaccine cycling?
 - Was litter cleanout too excessive?
 - -Were birds placed on new litter?
- Were birds treated with amprolium?
 - Amprolium treatment is discouraged with Coccivac, except in emergency situations until underlying administration or management problems can be corrected. If amprolium treatment is necessary, recommended use is at lowest dose level for 2 days only. Scenarios requiring treatment with amprolium include:
 - Increased number of resistant oocysts carried over from failing coccidiosis control programs.
 - An early extreme coccidial challenge associated with high litter moisture.



Amprolium treatment may slow or even stop full immunity development to Coccivac, which may result in a coccidiosis break later in the grow out cycle. Amprolium use should be considered a short-term "patch" until underlying issues can be corrected.

• Are house management practices (including lighting and feeding programs) encouraging litter-eating? - Litter eating birds may consume excessive oocysts and bacteria.

Necrotic enteritis breaks:

- Was excessive coccidial cycling found during monitoring program?
 - Scores of +3 or +4 in multiple birds constitutes excessive coccidial cycling
- Do the starter and grower rations contain an antibiotic with anti-clostridial activity? What is the inclusion rate and how long has the antibiotic been used?

- Some factors determining onset of necrotic enteritis include:
 - Is in-feed antibiotic in the correct feed?
 - Is in-feed antibiotic at correct level for *Clostridium* control?
 - Has antibiotic resistance to *Clostridium* developed?
- Was an in-feed anticoccidial included in starter or grower ration?
 - Early inclusion may disrupt vaccine cycling.
 - For hybrid (bioshuttle) programs, ensure in-feed anticoccidial is not introduced to birds earlier than 15 days.
- Wet litter. Was litter properly prepared between flocks?
 - High litter moisture favors *Clostridium* bacterial growth. Review bird density, litter type and depth, ventilation and ration formulation.
 - Litter amendments help reduce ammonia levels, reduce pH, creating a poor environment for clostridial species, and reduce stress on the bird.
 - -Windrowing litter between flocks may break the cycle of necrotic enteritis on repeat farms.
- Avoid rations with wheat, bakery meal, DDG's, barley or rye?
 - -These feed ingredient types can damage the intestinal mucosa and lead to necrotic enteritis.
- Ensure good quality fat sources are used?
 - Rancidity of fats, ethanol corn oil byproduct instability and poor-quality acidulated oils can lead to necrotic enteritis.
- Are appropriate enzymes included at sufficient levels?
 - Ensure diet appropriate enzymes are included to improve ingredient digestibility and minimize necrotic enteritis incidence.
- Monitor ration for ingredients containing high *Clostridium* spore counts? - *Clostridium spp.* is the pathogen responsible for necrotic enteritis.
- Does the region have high soil or water pH?
 High pH favors *Clostridium* bacterial growth.
- Did birds get too cool from 10-21 days (especially in ABF/NAE flocks)?
 - Cold birds are stressed and are unable to maintain body temperature. In turn, birds overeat beyond their digestive capability. This sends excess protein in the distal GI tract, which is known to cause clostridial blooms and potentially necrotic enteritis.
- Was feed presentation consistent during transition from starter to grower?
 - -Transitioning from crumbles to a pelleted feed, has the potential for accidental feed restriction, if birds do not readily accept the new feed presentation. This can also exacerbate litter consumption.
- Avoid potential brooding conditions or issues, which can cause consequential feed restrictions.

Pathogen Control

Pathogenic microorganisms such as viruses, bacteria and protozoa can be minimized in poultry houses by following good management practices.

- A good, sound biosecurity program should be developed and implemented to minimize the introduction of pathogens onto the poultry premises.
- Water treatments with buffered organic acids (such as citric, lactic, propionic, benzoic, tannic, acetic acids) and chlorine compounds have been shown to help minimize bacterial load. Water treatment products may be used throughout the grow-out cycle but discontinued 24 hours prior to water vaccination. For more information on setting up a water sanitation program, please consult Merck Technical Services.
- Maintain healthy immune status of flocks with sound vaccination programs against immunosuppressive diseases such as Marek's disease, infectious bursal disease, reovirus and chicken infectious anemia virus.
- Administration of vitamin and trace mineral supplements is recommended during the peak vaccinal cycling period.
- Administration of phytochemicals, probiotics, acids, prebiotics or competitive exclusion (CE) products may be helpful in maintaining a healthy and balance intestinal microflora. Prebiotics, probiotics and CE products are more effective when introduced to the bird as early as possible before the delivery to the farm.

Special Considerations for Coccivac-D2

Coccivac-D2 contains two additional species of *Eimeria* (*E. necatrix* and *E. brunetti*) which require additional life cycles to develop full immunity. **House conditions and attention to cycling must be maintained through at least 4 weeks of age.**

- Replacement pullets are placed at lower density than broilers.
 Floor space during brooding must be conducive to promoting controlled vaccine cycling
- Farm-to-farm management of replacement pullets is often variable.
 - Very dry litter conditions may prevent vaccine cycling
 - Very wet litter conditions (either throughout the house or just under bell drinkers) may promote excessive cycling.
- Large postmortem sessions to monitor vaccination in replacement pullets are impractical. Postmortem examination of *fresh* mortality and/or sex-slips is a good way to monitor cycling.
- If bloody droppings or increased mortality is observed, birds should be evaluated for *E. tenella*.
 - Blood filled ceca can be caused by Salmonella spp., histomoniasis and E. tenella
 - Use microscopic or histologic evaluation for E. tenella confirmation.

Other considerations

Feed Spray Application

When hatchery application of Coccivac is not possible or was accidentally not applied, feed spray application can be an effective alternative. Uniform ingestion of vaccine oocysts via feed spray is the goal. This includes proper application technique and overall brooding management. **Chicks must be comfortable to consume feed uniformly!**

Consult Merck Technical Services regarding timing of on-feed vaccination.

- **Do not** withhold feed or vaccinate chicks first thing in the morning. These procedures encourage overconsumption of feed by the most aggressive chicks, leaving no oocysts on the feed surface for the rest of the flock.
- **Do not** use medicated drinking water or water disinfectant 24 hours before, during, or 24 hours after, vaccination.
- Dilute Coccivac D2 at a ratio of 1,000 doses/400 ml of non-chlorinated water and add red dye.
- Mix vaccine well and place in clean garden-type pressure sprayer designated for vaccine use only. Keep vaccine agitated throughout vaccination procedure, to prevent oocysts from settling in the solution.
- Use half of diluted vaccine volume to uniformly cover surface of feed in feed lids, brooding paper and feed pans. All feed available to the chicks should be covered with vaccine, which darkens the feed surface (the feed should be damp, not wet).
- When coverage is complete, repeat process with remaining volume of diluted vaccine. Feed should be dry enough to allow vaccinator to observe feed darkening with reapplication. **Two complete passes over the feed will improve uniformity of coverage.**
- Allow chicks adequate time to consume vaccinated feed before placing more feed in feed lids, on the paper or in the pans.
- Post-vaccination reaction and response **should be monitored**.

If concerned regarding post vaccination reaction, please consult Merck Technical Services.

For additional information regarding special considerations for Coccivac-B52 or Coccivac-D2, please contact your Merck Animal Health Technical Service Team.

Coccidial vaccines have been successfully incorporated into coccidiosis management programs worldwide. They have also been successfully used as the exclusive coccidiosis management program in many antibiotic free and organic production companies. The vaccination program has proven successful under a wide variety of management and housing conditions, yielding the same farm-to-farm performance consistency as in-feed anticoccidial programs. These guidelines are designed to minimize farm-to-farm variation while maximizing the performance potential of Coccivac-vaccinated flocks.



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