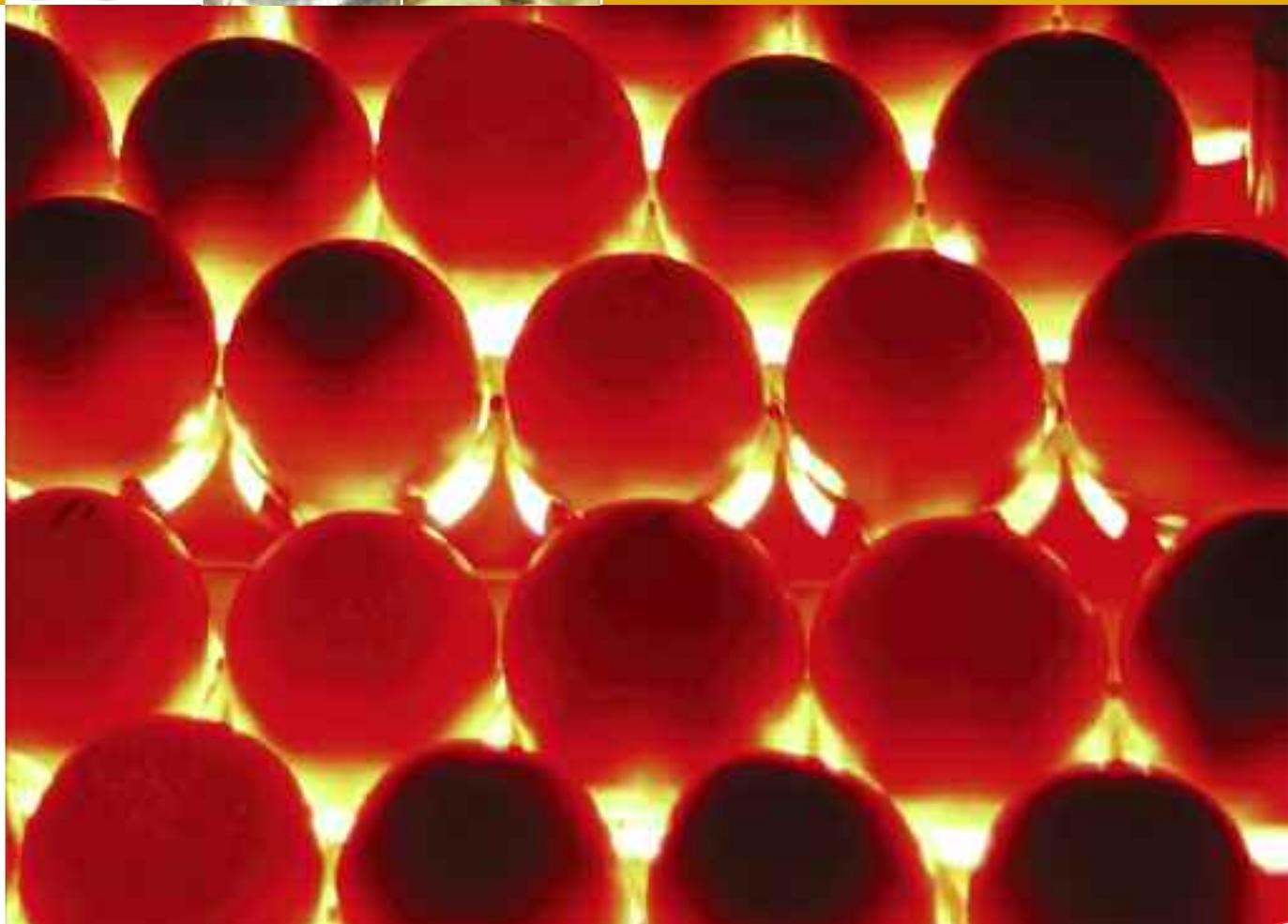


ASPERGILLUS CONTROL
IN HATCHERIES WITH CLINAFARM®



Introduction

Aspergillus: a worldwide hatchery problem

Aspergillosis is caused by a contamination with fungi of the genus *Aspergillus*, that may create severe economic losses in the poultry industry worldwide. *Aspergillus* is a fast-growing mold that only requires warmth, a little moisture and plenty of organic material to thrive. The fungi are found everywhere in the environment, from tightly closed jam jars to the sterile environment of an operating theater.

But it would be hard to find a more ideal breeding ground than the modern poultry production chain, hatcheries in particular.

Over the past few decades, modern hatcheries have undergone dramatic changes. Air-conditioning is standard in new hatchery construction, and A/C is a common addition when old hatcheries are upgraded. Vaccination techniques have changed: *in ovo* vaccination is nearly universal in US broiler hatcheries. Even chick transportation has evolved as modern air-conditioned chick transports have replaced the old converted school buses. Each of these changes present new challenges to biosecurity, sanitation and monitoring programs.

This brochure provides you with detailed information about *Aspergillus* and its consequences as well as tips and advice on how to effectively control this pathogen in hatcheries.



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1.1 What is aspergillosis?

The *Aspergillus* mold only requires warmth, moisture and organic material to proliferate. Its requirements are so modest that these fungi can be found everywhere in the environment, and its spores may disseminate over large distances. Disease due to *Aspergillus* is called aspergillosis.

The fungal pathogen

The most common *Aspergillus* molds found in poultry and game bird farming are *Aspergillus fumigatus* and *Aspergillus flavus*. *A. fumigatus* grows easily on organic material such as eggs, down and poultry litter, while *A. flavus* is more likely to grow on feed grains such as wheat, maize, oats and rye. Other *Aspergillus* molds, such as *A. niger*, *A. nidulans* and *A. versicolor*, are also seen in poultry production.

Aspergillus spores have a diameter of 2.5 microns, far too small to be seen by the naked eye. A small speck of dirt or fecal material on an egg can contain many thousands of spores which may each grow into a mature mold, producing spores and spreading contamination. For example, a single gram of chick fluff may contain up to 190,000 mold spores.

Decreased hatchability

One of the consequences of aspergillosis in a hatchery is decreased egg hatchability, due to environmental infection of the egg. Embryos die at about 16 days of incubation, as a result of spores entering the egg via a porous shell or a thin hairline crack.

This does not necessarily mean that the eggs were contaminated at the hatchery: the spores may have arrived with the eggs.

Nevertheless, the hatchery is seen as the main proliferation site. There are constant opportunities for entry and production of spores and all hatcheries undergo a continual or seasonal challenge from *Aspergillus*.



Fumigatus



Flavus



Niger

1.2 The symptoms

Newly hatched chicks: respiratory and nervous signs

In hatched chicks, infection occurs after the inhalation of large numbers of spores from a contaminated hatchery environment. Chicks are particularly vulnerable during the first three days of life, as their respiratory system is too immature to fight off the infection. Chicks often become infected immediately after hatching. This leads to mortality in the first ten days after hatching. When inhaled by a newborn chick, the spores will cause lesions of the respiratory tract, leading to breathing difficulties. Necropsy of infected chicks reveals yellow clumps of fungi in the trachea, air sacs and lungs.

Chicks may also have difficulty walking if the fungus has gained access to the central nervous system. High early mortality may occur and surviving chicks do not grow well. Birds infected subclinically may show no obvious signs but will have reduced weight gain, increased feed conversion and/or higher condemnation rates at the slaughterhouse.

Acute brooder pneumonia: high mortality in hatcheries

The acute form of aspergillosis, also called brooder pneumonia, is mainly seen in hatcheries and on broiler farms. Over 90% is hatchery related. It is characterized by severe outbreaks among young chicks, with high mortality rates (up to 30%) in the first

10 days of life. Respiratory signs include dyspnea, yawning, rapid breathing and stretched necks. Secondary infections due to bacteria such as *E. coli* or *Salmonella*, causing systemic disease, are not uncommon. Surviving chicks will often show a lack of performance later in life.

The chronic form: more common in adult birds

The more chronic form of aspergillosis is usually seen in broilers from 4–5 weeks of age and in breeder farms, especially in turkeys. The disease is most commonly brought on by contaminated litter, particularly if thick, damp and matted with feces. Feed or other organic material may also be a source of contamination. The chronic form is often observed as a secondary infection in already weak or diseased birds.

Apart from classical respiratory symptoms seen in chicks, there is a spread to other organs, and *Aspergillus* may cause a systemic infection, dermatitis, osteomycosis, ophthalmitis, encephalitis and polyserositis, including arthritis. Typical post-mortem findings are the granulomatous nodules in the lungs and air sacs. These 'aspergillomas' contain inflammatory cells and fungal hyphae. Plaques of caseous and mucopurulent exudate may be seen on the trachea and on air sac membranes at necropsy.



1.3 Diagnosis

Aspergillus spores are present everywhere in the environment, and are carried on air currents until they come into contact with an organic growth substrate. The prevention of aspergillosis in hatcheries requires appropriate monitoring of the presence of spores in the environment. Samples may be taken at various locations and from various sources.

Air sampling

Routine sampling of the air in hatcheries is a simple and inexpensive method to test for the presence of *Aspergillus* spp. Agar plates with specific growth media such as Sabouraud-dextrose agar are readily available. These media may include an antibiotic such as chloramphenicol to reduce the contamination of the plates by airborne bacteria. Cycloheximide should be avoided as this may inhibit *Aspergillus* growth.

Plates are placed open in the area to be sampled, with the lids next to them, facing downwards (to avoid the collection of dust and spores). After ten minutes, the lids are replaced and sealed to the plates with adhesive tape. The sampling location is marked on the bottom of the plate, which is then incubated, upside-down, to reduce condensation on the agar. After 36-48 hours, the mold colonies may be counted.

Aspergillus fumigatus, the most common of the *Aspergillus* molds found in hatcheries, have a blue-green appearance with a white border. The base of the mold growth, on the underside of the plate, is colorless or yellowish white. Immature *Aspergillus* molds are small and white. If these are found, the plate should be incubated for another 5-6 hours after which growth will be better recognizable.

Simultaneous laboratory tests may help you recognize *Aspergillus fumigatus* by sight with reasonable accuracy. However, even if molds of another, non-pathogenic variety are found, this indicates that environmental conditions in hatcheries are ideal for mold proliferation—of any kind.



The egg test: an inexpensive alternative

As an alternative, infertile eggs may be used instead of agar plates. A hole (about .4 inch diameter) is made in the top of an infertile egg, which is placed hole-upwards on a plastic egg tray and left for one hour in the area to be sampled. Label the egg according to date and sample area and place it together with other such sample eggs in a tray. Seal the tray in a polythene bag before incubating to avoid the risk of contamination. Accurate colony counts are not possible with this method but it provides an inexpensive alternative to spot check sampling.



Egg necropsy

The presence of *Aspergillus* molds can also be detected in eggs or birds. Egg necropsy is often carried out routinely to check flock fertility, embryo mortality, malposition or malformation. In order to determine the degree of fungal contamination, hatcheries should perform egg necropsy on unhatched eggs every hatchday. At day 21, unhatched eggs from a few baskets per hatcher should be opened to examine the air cells for mold growth. Ideally, eggs from each supplying breeder flock are included. Before breaking, the eggs should be closely examined for cracks or porosity of the shell.

Eggs found to contain small typical greenish-blue molds due to *A. fumigatus* at necropsy were probably contaminated 3 to 5 days earlier, most likely during transfer or *in ovo* vaccination. This mold growth is easily visible in unfertilized or early dead

embryonated eggs. If the egg was contaminated at an early stage, e.g. at breeder farm level, the eggs will be very light following evaporation of the egg contents due to a porous or hairline-cracked shell. The air chamber is large and may be filled with blackened spores.

If embryonic death occurred, contamination with *Aspergillus* and environmental bacteria will have led to breakdown of the egg content, leading to a putrid debris in the egg.

All eggs containing mold should be recorded to monitor the contamination source and trends. Necropsy should be carried out outside the hatchery to prevent contamination if infected eggs are found. A protective mask should be worn.



Do's and don'ts of air sampling

Do:

- Sample regularly to follow contamination trends.
- Sample in the same places under the same conditions to allow comparison.
- Sample hatchers only when clean, empty, dry and at working temperature, in order to check the efficacy of hygiene procedures.
- Have a laboratory test carried out initially to identify *Aspergillus* molds.
- Inform all hatchery operators of the time and place of sampling to avoid breakage or disturbance of plates.
- Check results from agar plates away from the hatching area to avoid contamination.
- Wear a mask when examining the results, as *Aspergillus* spores are potentially harmful.
- Record the results and take pictures for easy comparison.

Do not:

- Place sample plates in areas of activity, near doorways or ventilators as the air turbulence may influence the colony count.
- Sample during washing or disinfection procedures, as disinfectants and detergents may have an impact on the agar and subsequent counting or identification.



Eggshell sampling

The direct sampling of eggs allows an early warning of a build-up of *Aspergillus* at the breeder farm. Although this type of sample does not give accurate colony counts, it may be indicative of a problem at breeder farm level, in particular if egg samples from the same flock are consistently positive for *Aspergillus* growth.

Eggshell sampling is easy: a cross is drawn with a felt tip pen on the base of an agar plate, dividing the plate in four quarters. Remove the lid of the plate and lightly touch the tops of 4 eggs on the growth medium, one on each segment. Note the name of the flock on the base of the plate and incubate as described previously.

Surface sampling

A sterile swab is used to take a sample of dust from a surface such as a ventilation grill or hatcher exhaust, preferably when clean. Not only will a dirty area almost invariably prove positive, but sampling of clean areas allows you to check the efficacy of cleaning procedures. Although this test will not give accurate colony counts, it is indicative of hygiene measures.

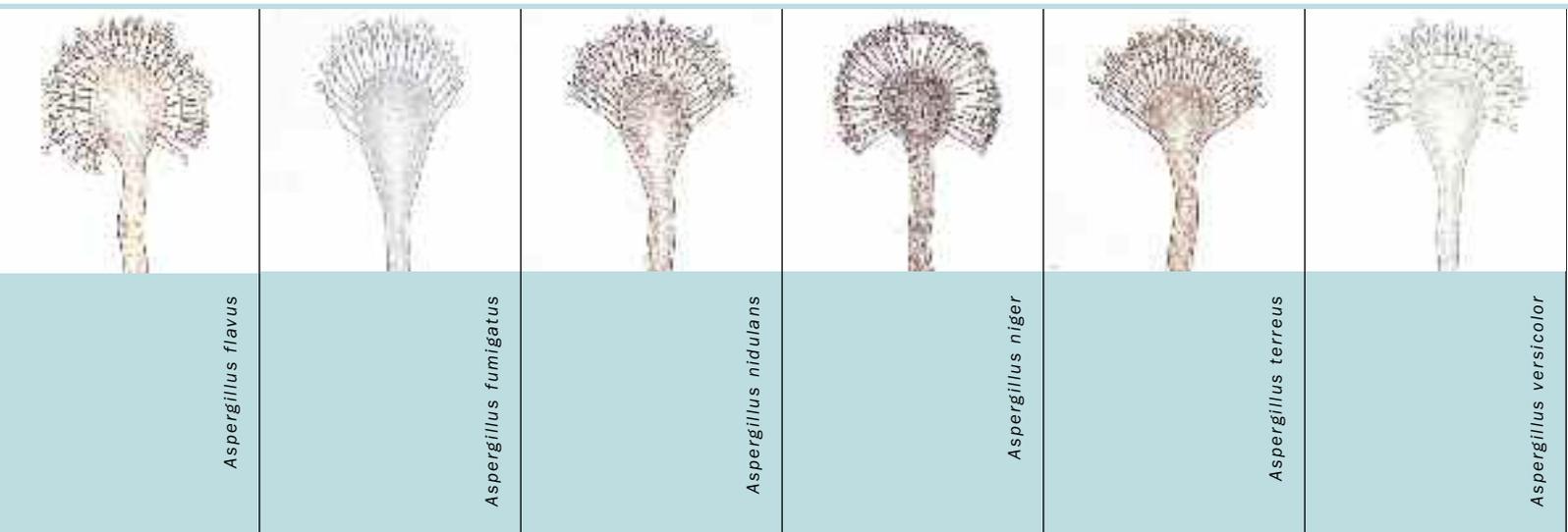
After sampling, an X or W is drawn with the swab across the agar surface of a plate. Mark the position of sample area and incubate.

Mold growing on certain surfaces such as wood or cardboard may not always be easy to recognize, and should be swabbed and cultured for identification.

Under the microscope

Lesions of dead birds can be sampled and sample material can be cultured and examined under a microscope, as well as samples from eggs or environmental cultures. Under the microscope, the septate hyphae and typical, dandelion-like conidia of *Aspergillus* may be visible. The hyphae can be stained using lactophenol cotton blue. Alternatively, first a potassium hydroxide solution may be used before staining. When using necropsy material, *Aspergillus* conidia with spores will only be found in body compartments open to the outside air, such as the respiratory system.

The various *Aspergillus* species can be identified on the basis of the microscopic and cultural properties.



1.4 *Aspergillus* and aflatoxin

Aspergillus can grow on stale, damp poultry feed and produce the mycotoxin known as aflatoxin. When feed contaminated with aflatoxin is consumed by the breeders, this may lead to immunosuppression, a drop in egg production and reduced fertility/hatchability of the eggs.

Aflatoxin production may occur at the feed mill or even earlier, in grain stores on the farm of origin. Feed may also become contaminated in bulk feed bins on the breeder farm if these are not emptied prior to each new feed delivery. The design of certain bulk bins may cause feed remnants to stay behind in nooks or ledges, thereby contaminating the fresh feed delivery.

Conditions in feed bins are ideal for *Aspergillus* growth and sporulation, as they are subject to temperature changes (hot during the day, cool at night) and moisture due to condensation. Bagged feed and additives should be stored in a clean, dry area.

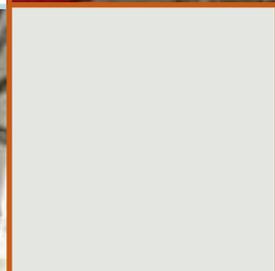
1.5 Impact on the production chain

The main origin of *Aspergillus* contamination at hatchery level is the breeder farm.

Ultimate control begins at the breeder farm with good litter control, nest box hygiene, more frequent egg collection and careful egg grading, removing all damaged and dirty eggs. Eggs should be stored under hygienic conditions and be transported to the hatchery in clean vehicles, driven with care.

Poor hygiene or insufficient antifungal treatment will perpetuate an *Aspergillus* contamination in the hatchery. Hatcheries in turn are a potential source of aspergillosis problems at the broiler farm. Poor performance or even clinical signs and mortality may be seen in young broilers that were infected as chicks.

Hygienic measures and antifungal treatment at all levels are therefore essential.



Chapter 2

ASPERGILLUS AND ASPERGILLOSIS: IN THE FIELD

2.1 Hatcheries: the perfect breeding ground

It would be hard to find a more ideal breeding ground for *Aspergillus* than the modern poultry production chain, in particular at hatchery level, where moisture, temperature and nutrients create an ideal environment for the growth of *Aspergillus*.

Spores are easily transported by light air currents (wind, ventilation...). Furthermore, the concentration of hatching eggs and chicks lead to a permanent exposure to significant numbers of fungal spores and recontamination.

The incubator: ideal conditions for *Aspergillus*

Aspergillus requires no specific conditions for growth or germination. The spores thrive in a wide range of temperatures (54–149°F), moisture and pH. Optimal growth is achieved at temperatures of 98.6–113°F and high humidity. In other words, an incubator is an ideal growth environment.

Growth and sporulation require a cycle of warm 'wet and dry' conditions: wet conditions for growth and dry for sporulation. Together with a growth medium such as egg yolk or other organic material (wood, cardboard, chick fluff), an ideal breeding environment for *Aspergillus* is created. As a result, storage areas should be monitored frequently. Efforts should be made to minimize storage of cardboard boxes or wooden pallets and dollies within the hatchery.



Ventilation systems: difficult to clean

The 'wet-dry' cycle is also maintained by the chick take-off or pulling (dry) followed by washing and cleaning of the hatcher (wet). Combined with active ventilation, this may explain the often high *Aspergillus* proliferation in hatchery ventilation systems. As they are often difficult to clean routinely, ventilation, filters, shafts and exhausts are a continuous source of recontamination.

Evaporative coolers

These devices, often roof mounted, use a pad which has cool water pumped across and the hot air from outside the hatchery is pulled through the pad. This reduces the temperature of the air by trapping the heat of the air in the pad. Any organic debris can be trapped inside the pad.

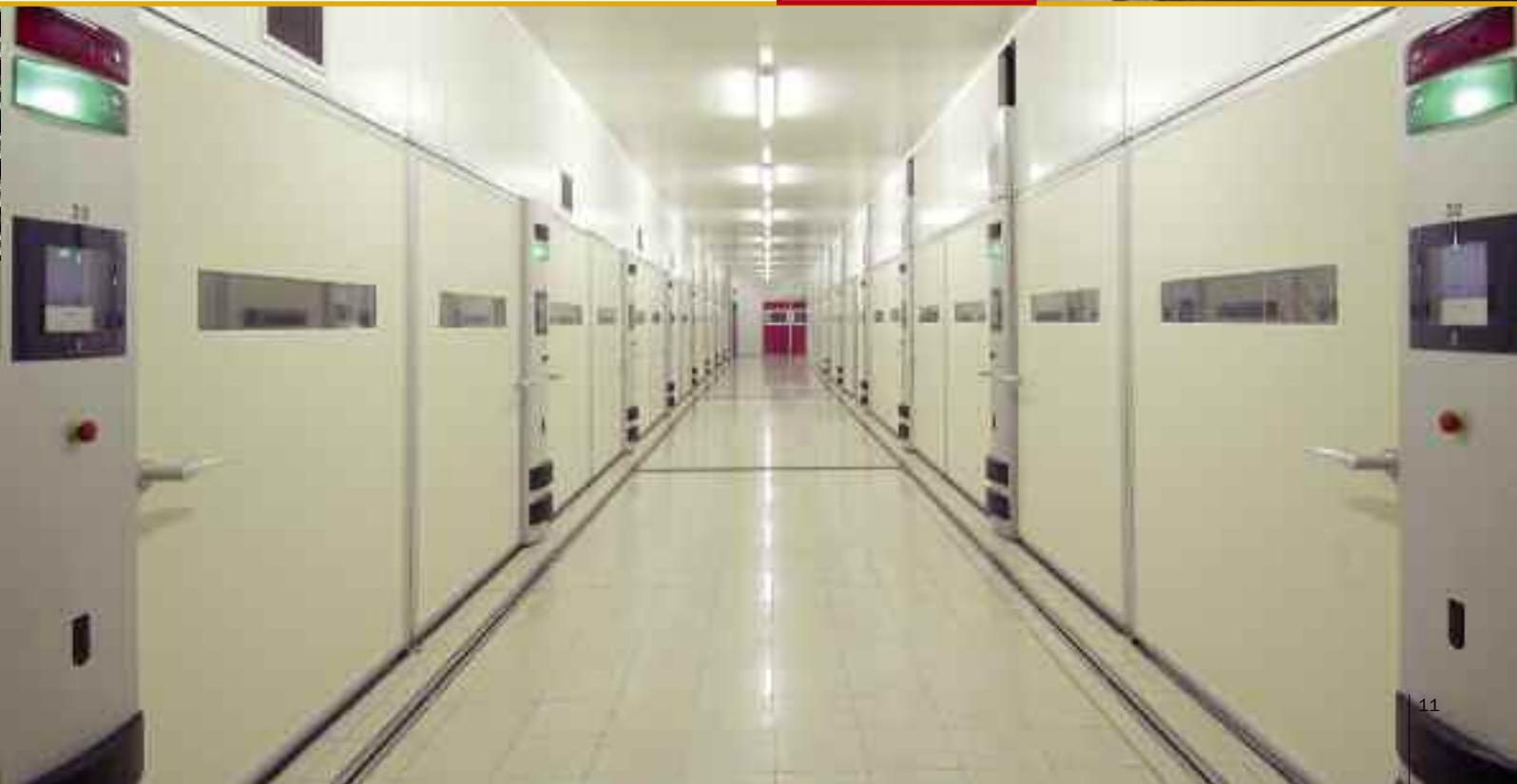
At night or during periods of cooler weather, these coolers are often not used. Consequently, this allows the pads and any organic matter to dry out. Thus perpetuating the wet-dry cycle needed for growth and sporulation of *Aspergillus*.

Air Conditioning

Air conditioning has presented hatcheries with new challenges for sanitation. Filter maintenance is critical, as is cleaning of the coils (especially the inside coil), the ductwork, the directional grill and especially, the condensation pans.

External sources

Although less common, outside sources of *Aspergillus* are also possible. These include pollinating trees in the area, dust from harvesting corn or other field crops, dust from feed mills or wood processing plants. Even cutting the grass around a hatchery is not without risk! Spores can be drawn into the hatchery via the ventilation systems and contaminate the internal ventilation units.





2.2 the Modern Hatchery: a portrait

The development of modern hatcheries in the past few decades has been enormous. Chick output from hatcheries has risen from a typical average of 700,000 to 4 million per week in some hatcheries.

Production schedule

The production schedule has changed, too. While thirty years ago, most hatcheries would hatch two days per week, transfer the eggs on two other days, leaving a day for cleaning and disinfection, modern hatcheries will hatch 4 to 6 days per week. This also means that all major operations, such as the setting of eggs, the transfer from setter to hatcher and the take-off or 'pulling' of chicks, take place on the same days, increasing the risk of cross-contamination.

US Hatcheries – Multi-stage setter challenge

Although the primary breeders and some new broiler hatcheries have been constructed with single-stage incubation technology, most of the US broiler hatcheries still depend upon multi-stage incubation systems. Multi-stage units may never be completely emptied for cleaning and disinfection over several years of use. Many efforts have been made to attempt cleaning and disinfection without complete emptying of the machines. These include disinfectant fogging of the setter room and disinfectant fogging of the incubators themselves. Fogging with disinfectants creates a problem with humidity that can be difficult to control.



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Modern equipment and design

Construction of US hatcheries ranges from old metal buildings with fiberglass insulation to more modern pre-cast concrete construction. Both designs have strengths and weaknesses. For example, metal wall and ceiling panels are non-porous and can be cleaned efficiently, but the fiberglass insulation behind the wall can become contaminated with bacteria or mold. Pre-cast concrete designs are solid but the concrete floors, walls and ceilings may be porous and may harbor mold or bacteria.

Many hatcheries have plenum chambers to exhaust the air laden with dirty fluff from hatcher, allowing easy and efficient cleaning. Most hatcheries have installed positive air pressure to prevent dust from moving into clean areas. However, this system obviously only works if all doors in a hatchery are kept closed.

Increased automation: a potential source of contamination

Increasing manpower costs have triggered a wave of automation in hatcheries. Operations such as egg transfer, candling, *in ovo* vaccination, chick separation and counting have largely become automated. This also means that all eggs or chicks pass through the same equipment, which may become a source of infection if they are not regularly and effectively cleaned and disinfected.

Increased vigilance is required as badly adjusted transfer machines may break eggs or cause hairline cracks during handling, while the vacuum heads, pipes and filters may become contaminated, spreading mold and bacteria from egg to egg.

While candling machines effectively identify and remove infertile eggs at transfer, the waste container may become a potential source of contamination if not strictly separated from the transfer area.

Chick separators may stir up large volumes of dust, while chick counters may cause stress and/or damage to the chicks.



***In ovo* vaccination: optimal hygiene required**

Automated *in ovo* vaccination has dramatically increased the speed and efficiency of vaccination in hatcheries. However, it may also be a potential source of *Aspergillus* infection, in a contaminated environment, as the opening created by vaccination may allow spores to enter and grow on the air cell membranes. *In ovo* vaccination should therefore be carried out under strict hygienic conditions: clean, uncontaminated air, aseptic vaccine mixing procedures and strict cleaning and antifungal disinfection procedures.

Most *in ovo* vaccination machines have specialized apparatus and procedures for cleaning their own integrated transfers and the manufacturer's recommendations should be strictly followed. The waste container should always contain an anti-fungal disinfectant.



Main *Aspergillus* risk factors at hatchery level:

- contaminated, cracked or dirty eggs coming in from the breeding farm
- contaminated ventilators and filters
- badly adjusted transfer machines (damaged eggs, source of contamination)
- failure to follow proper *in ovo* procedures
- contaminated vacuum heads and pipes
- discharge container for rejected eggs
- badly adjusted chick separators/counters (dust and stress)
- reuse of insufficiently cleaned equipment
- contaminated cardboard or wooden material
- poor biosecurity (insufficient hygiene, open doors...)
- poor hatchery design: no separation between 'dirty' and 'clean' areas
- the use of disinfectants with limited antifungal activity
- inadequate cleaning of conveyor belts, especially fiber belts that are particularly difficult to clean



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2.3 The economic impact

The main consequences of aspergillosis contamination are major economic losses at hatchery level due to lower hatching rates and chick mortality. Chicks that were contaminated early in life will turn into broilers with a decreased performance, a higher mortality rate and a possible source of environmental contamination.

The overall cost of a major *Aspergillus* infection is due to:

- Increased embryo mortality (especially at day 16), causing decreased hatch
- Early chick mortality (up to 50% in worst cases)
- Weakened viability of chicks, immunosuppression
- Poor weight gain of broilers
- Increased mortality during growth period
- Increased cost to overcome infection
- Loss of grower confidence in chicks

The benefit from Clinafarm® disinfection

Although fortunately a full-blown aspergillosis outbreak is not very common, most hatcheries will suffer from the subclinical effects of aspergillosis in broiler flocks. This may go unnoticed in the absence of high mortality rates, but poor growth in a flock, lack of vitality and uneven growth are all signs of a subclinical aspergillosis infection.

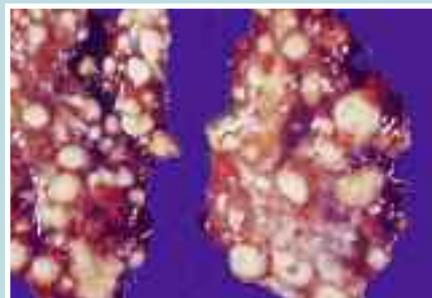
Aspergillus contamination has a significant impact on the technical and financial results of a hatchery, and a Clinafarm® disinfection program is a justifiable investment. The return on investment of a Clinafarm® program can easily be achieved by a 0.5% improvement in hatchability.

No cure, only control

Treatment of the disease is virtually impossible and serves no economic value to commercial producers. Once a hatchery is contaminated it is impossible to get rid of *Aspergillus* completely due to the easy growth of the pathogen and the favorable growing conditions at the hatchery. While antifungal treatment will allow you to control the problem, it is highly unlikely that it can be removed completely and permanently. However, strategically designed sanitation programs should be able to effectively control the environmental infection pressure and prevent substantial economic losses. Therefore, this should be a strategic focus of hatchery managers.



sick/weak broiler



Aspergilloma



respiration problems



chick mortality

2.4 Case histories

case 1

Poor hatchery management = poor broiler performance

A hatchery had noticed a higher than average early mortality of chicks placed. Managers on the growing farms were also complaining that the finished birds were not achieving their target growth weights and showed a lack of uniformity.

Sampling at the hatchery showed that egg storage, incubation rooms, hatcher rooms and chick handling rooms were all heavily contaminated by *Aspergillus*. Investigation also revealed a lack of hygiene (insufficient cleaning and disinfection) and biosecurity (doors left open between 'dirty' and 'clean' areas). The hatchery manager explained that this was necessary as there had been problems with air availability for the hatcher.

The chick holding room was also used for box storage where new chick and egg boxes were stored on wooden pallets prior to assembly. Investigation revealed that the boxes at the back had been stored for several years, with new deliveries stacked in front. There were also large quantities of damp chick fluff under the pallets. This was heavily contaminated with *Aspergillus* as were the old damp boxes.

Every time chicks were stored in this room, fans were used to circulate air and cool the chicks. Coupled with the open doors, this enabled *Aspergillus* to colonize the whole hatchery.

Although it was impossible to quantify the losses, the growing farms had been complaining for a long period about mortality and decreased performance. However, it is certain that an extra box store and an appropriate ventilation system would have largely covered the losses.



case 2

A small mistake with major potential consequences

A well-managed, modern hatchery plant with an efficient ventilation system and single-stage incubation had noticed a rise in *Aspergillus* counts during routine sampling. This coincided with a sharp rise in temperature during a particularly hot summer. Examination of unhatched eggs showed a small increase in air cell mold.

On hot days, the hatchery would remove some of the filters in the ventilation system to allow for better airflow and cooling. This had been done for many years and had never caused a problem. Furthermore, recorded sampling results showed that no increase in mold counts were noted previously following the dates when filters were removed. The entire ventilation system was cleaned weekly.

Further investigation revealed a dumpster outside the hatchery walls, containing macerated hatchery waste from the previous days' hatch. The dumpster was 10 feet away from the hatchery air inlet grill.

Normally, the dumpster was situated on the other side of the hatchery close to the macerator and the exhaust air outlets. Due to increased waste removal charges, the waste was only collected every other day instead of daily. To avoid a nuisance due to the smell of decomposition, it was decided to move the dumpster, once full, to the shaded side of the hatchery—where the air inlet happened to be. This was done in particular on hot days—the same days the filters were removed. Sampling of the dumpster showed very high contamination due to the waste company not washing and disinfecting the empty dumpsters before delivery to the hatchery.

Due to the good management practice of regular sampling and record keeping, the mistake was quickly rectified before a major problem could occur.



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case 3

How minor equipment malfunction jeopardizes healthy eggs

A hatchery which used egg necropsy noticed an increase in the incidence of mold on egg air cells. There was also an increase in eggs with bacterial contamination (rotten eggs). It seemed to be worse in particular trays. However, it did not appear to point to a problem with particular setters, hatchers or even particular flocks, it seemed to be completely at random. Nothing gave a clue as to why they were seeing a random increase in *Aspergillus* and bacterial counts. The on farm egg stores were checked and samples taken, these gave positive results but as *Aspergillus* is regularly found in the dust on poultry farms it was not thought to be the cause of the problem as the *Aspergillus* has to penetrate the shell of the eggs to cause air cell growth.

Also it was noted that many of the eggs were grossly contaminated with *Aspergillus* which pointed to contamination on farm or early in incubation. Again egg storage was examined. The farm egg stores were fairly primitive, no refrigeration or humidifiers were used, the egg stores were insulated and eggs were collected from the farms on a daily basis during the summer period when the problem was noted. The daily collection during summer was to get the eggs cooled as rapidly as possible using the refrigerated egg stores in the hatchery.

During sampling of the hatchery egg store it was noticed that there was a large wet patch on the floor in front of a humidifier. This had not been noticed during previous checks as the area had been filled with trolleys of eggs at those times. Examination of the humidifier showed that the atomization was not working properly due to a build up of calcium carbonate and the humidifier was actually spraying a fine spray of water over the warm eggs which had been brought from the farm. The position of eggs in the store was entirely random which meant that eggs from a different flock would be in this area every few days. These eggs fresh from the farm and very warm were being cooled to 61°F rapidly on entering the store and rapid cooling creates contraction, which has a suction effect. This combined with the wetting of the egg surface was causing both *Aspergillus* spores and other micro-organisms to be drawn through pores in the egg shells (particularly affecting eggs with porous shells) contaminating the eggs. Thirty minutes of maintenance work on the humidifier cured a problem that took weeks to identify.

case 4

How a cheap solution led to major losses

A hatchery using *in ovo* vaccination found mold in a large number of vaccinated eggs. The *in ovo* vaccination equipment and integrated transfer were sampled on a regular basis and were not suspected. The hatchers were then sampled and showed either nil or very light contamination. Attention was then directed to the transfer room where *in ovo* vaccination and the transfer of eggs was carried out simultaneously with transfer only of non injected eggs using an old vacuum transfer machine.

Sampling of the ventilation system was negative, but as soon as work started in the transfer room, sampling showed very high spore counts. Furthermore, it was revealed that the transfer head of the old machine had developed a vacuum leak the previous year, causing eggs to drop. The leak had been fixed by an operator by sealing the vacuum head permanently to its mounting plate using a silicone sealant. When cleaning the machine, the staff would just wash the vacuum cups at an angle using a pressure washer, leaving the transfer head *in situ*.

After removing the transfer head from the mounting plate it was found to be full of *Aspergillus* mold growing on egg yolk which had been sucked into the transfer by the vacuum. Every time this machine was used, it filled the transfer room environment with spores.



3.1 Clinafarm®, a unique fungicidal disinfectant

Even when strict sanitary precautions are in place, hatcheries will still be confronted with *Aspergillus*. A strong, specific disinfectant against this fungus is therefore required. From the wide range of safe antimycotics developed by Janssen Animal Health and distributed by ISP, imazalil was chosen as the most appropriate fungicidal disinfectant.

Imazalil has a very high activity against the *Aspergillus* fungi and its spores, and is therefore highly suitable as a fungicidal disinfectant. A spray formulation and a smoke generator are available, which may be used separately or in combination, depending on the circumstances.

Two presentations: spray and smoke

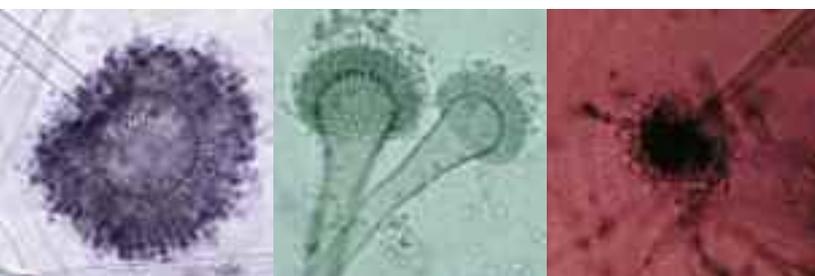
Clinafarm® EC is particularly suited for the spraying or fogging of large surfaces such as walls, floors and tools. Clinafarm®

EC is an emulsifiable concentrate containing 15% imazalil (5.3 oz/Qt). When mixed with water, Clinafarm® EC forms a stable micro-emulsion. It has a high bioavailability for the fungi and spores and a potent activity due to the low particle size of imazalil. The product's excipients have been selected to improve contact with surfaces, mold and spores, while the oil-based carrier provides a long-term effect—even in areas with high organic content.

Clinafarm® Smoke Generator is a ready-to-use imazalil smoke generator for fumigation of well closed rooms, laboratories or in small places such as incubators and ventilation ducts. Clinafarm® Smoke Generators contain .18 ounces of imazalil. Clinafarm® Smoke Generators may be stored for up to 3 years in a cool place.

3.2 Fungicidal and anti-sporulant

Clinafarm® is a disinfectant with both fungicidal and anti-sporulate properties, especially suited to combat aspergillosis. Many products claim a broad-spectrum efficacy against microorganisms and molds. However, they all have weaknesses when used in the hatchery environment.



Clinafarm®: portrait of an ideal fungicide

- Highly effective against all stages of *Aspergillus* (mold and spores)
- Product of choice for aspergillosis (1,000 times more potent than thiabendazole)
- Use in the hatchery environment does not affect the quality, incubation or viability of the eggs
- Specially formulated for environmental use and on surfaces in hatchery plants
- Can be used as a smoke, spray or fog
- Non-corrosive to equipment
- Shelf life: 5 years for the spray formulation, when stored correctly—3 years for the smoke generator
- Compatible with most detergents and other common disinfectants
- A strong long-lasting effect
- High vapor phase activity
- Specially suitable for controlling fungi in hatcheries



For example:

Formaldehyde: not effective against mold or spores in normal applications under common hatchery conditions, highly toxic to both humans and animals, in particular to 3 to 8-day old embryos. Carcinogenic.

Quaternary ammonium: inconsistent activity against aspergillosis, ineffective against spores.

Iodine-based disinfectants: not very effective, possible adverse effects on hatchability in high concentrations.

Chlorine-based disinfectants: not effective against mold, unstable during storage and use. Highly corrosive. No residual effect. Should not be fogged.

Phenolics: variable activity against molds and spores. Incompatible with certain detergents and other treatments. Toxic. Cannot be fogged.

Glutaraldehyde: ineffective against aspergillosis and spores, moderately toxic. Cannot be fogged.

Hydrogen peroxide: highly corrosive to metals, cannot be fogged.

Peracetic acid-based disinfectants: inconsistent effect, corrosive, should not be fogged.

Ozone: highly corrosive, should not be fogged.

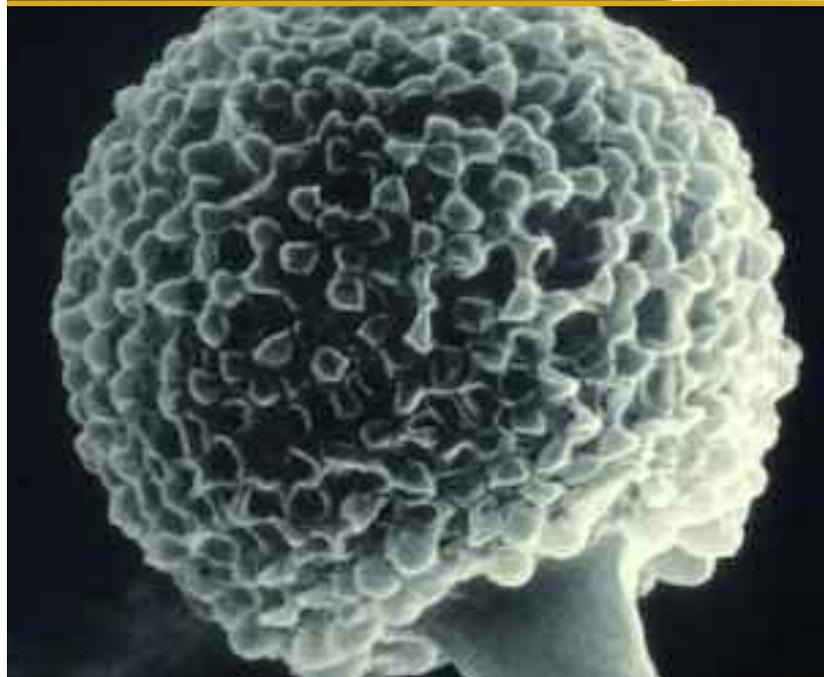


3.3 Efficient, safe and easy to use

Mode of action

Imazalil selectively prevents the formation of ergosterol, which is an essential part of the fungal cell walls.

At extremely low concentrations (1µg per mL), Clinafarm® is capable of inhibiting the growth of the *Aspergillus* type fungi. Clinafarm® is both fungicidal and anti-sporulant.



Active in the vapor phase

To test the vapor phase activity of imazalil, a small paper disk impregnated with imazalil was placed inside the cover of an inverted agar plate (1), allowing no direct contact between the imazalil disk and the culture medium. Any inhibitory activity observed is entirely due to the gas phase.

As a comparison, another imazalil paper disk was placed directly on the culture medium of an inverted agar plate (2). Here, the observed activity is due to diffusion.

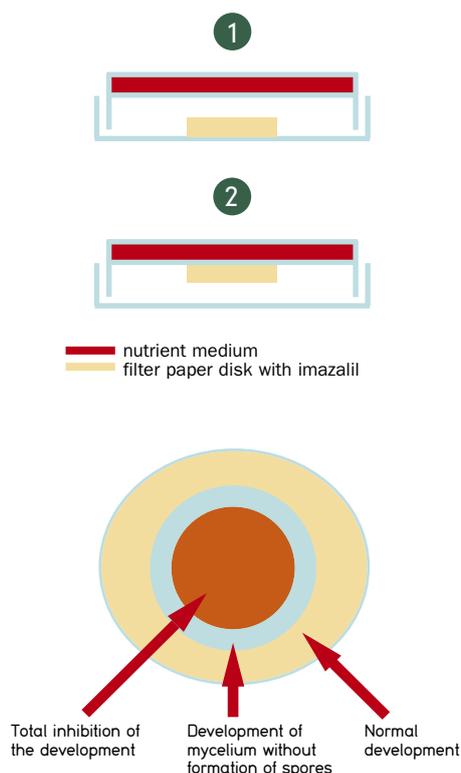
Both plates were inoculated with *Aspergillus*. After incubation, both plates showed a central area in the culture medium with total growth inhibition of the fungi and a secondary area where the mycelium had developed, but no spores were formed.

Even in the absence of direct contact, the gas phase activity of imazalil has a strong activity inhibiting mycelial growth and spore formation.

A thousand times more active than thiabendazole

Imazalil is more than a thousand times more active against *Aspergillus* than thiabendazole. It is also more effective against dermatophytes. The growth of most fungi was completely inhibited at concentrations of 1 to 10 mg/l. It is even active against yeasts and, to some extent, against gram-positive bacteria.

Imazalil, vapor phase activity



Comparative activity of imazalil and thiabendazole against fungi (minimum inhibitory concentrations in µg/mL)

	Imazalil		Thiabendazole	
	1	(0.1)	1	10
<i>Aspergillus fumigatus</i>				

(*) at 1 µg/mL: complete inhibition

(**)(): partial inhibition

A long-lasting effect

The excellent residual activity of imazalil was demonstrated in an experiment where agar plates were first exposed to Clinafarm® Smoke Generators (1 g / 15 m³). After 30 minutes, they were inoculated with *A. fumigatus* and incubated. The exposed plates showed 99.9% inhibition of growth.

The efficacy of the spray formulation was confirmed in a commercial hatchery. In this hatchery, air samples revealed the presence of *A. fumigatus*. Imazalil was applied as a spray (20 mg/m²), after which agar plates were placed at various locations throughout the hatchery. All plates remained negative for growth after incubation for two days. On day 3, the plates were inoculated with *A. fumigatus*, but no growth developed.

This shows that imazalil in Clinafarm® has a strong long-lasting effect, irrespective of the presentation (in the smoke as well as in the spray formulation).

3.4 Clinafarm® EC, a user's guide

For large areas, surfaces and equipment

The Clinafarm® EC formulation contains 5.3 ounces imazalil per quart and has an excellent wetting effect on the water repellent spores, giving better contact between the product and the spores. Clinafarm® EC is particularly suited for fogging large areas or for spraying onto walls, floors and equipment. It is an oil-based formulation that sticks to surfaces and remains effective for a long time. Clinafarm® EC is less suited for small and confined spaces such as containers or incubators, or in areas that should not get wet, such as laboratories. Clinafarm® EC is also less suited for very high rooms, so in these cases Clinafarm® Smoke Generators should be used.

Dilute before use

Before use, the concentrated form of Clinafarm® EC should be first diluted one hundred times with water: 1.25 fl. oz. Clinafarm® EC to 1 gallon of clean water. Dilute only enough product for one day's use at a time. The diluted product may have decreased potency after 24 hours. The diluted product should then be sprayed at a use rate of ½ fluid oz of diluted product per 150 cubic feet. For optimal effect, the water should be warm (110° F). Spraying or fogging may be carried out with any type of spraying device.

Spraying or fogging

Diluted (1/100) Clinafarm® EC solution may be may be sprayed or fogged at the rate of ½ fluid oz per 150 cubic feet.

Effective, non-corrosive, highly compatible with other products

Clinafarm® EC is highly active against *Aspergillus*. Its activity is not influenced by the hardness of the water used to prepare the dilution. It is effective at temperatures ranging from 40–110°F. At temperatures of 77°F or higher, such as in incubators, an increased activity against *Aspergillus spp* is noted. It is non-corrosive to equipment and compatible with many other disinfectants, ectoparasiticides, insecticides and detergents.

Safe when used according to direction

Clinafarm® EC can be safely diluted with appropriate safety precautions such as goggles (or a face shield) and gloves. Treated areas should not be re-entered for two hours after treatment.



3.5 Clinafarm® Smoke Generator, a user's guide

For confined spaces and less-accessible areas

Each Clinafarm® Smoke Generator contains 5 g of imazalil, which is released as smoke by a special combustion process. A single generator is sufficient for an area of 400 to 500 cubic feet. The ready-to-use smoke generator is highly suitable for the disinfection of tightly closed rooms, such as laboratories and incubators, or transport vehicles. Furthermore, it can be used in less accessible locations such as ventilation and air-conditioning systems. No special spraying device is required.

Clinafarm® Smoke Generator is less suitable for open areas or the disinfection of walls and floors. In these cases, Clinafarm® EC is more appropriate.

Quick and easy

Before lighting the smoke generator, the room should be well closed for maximum effect: close doors and windows, seal slits and cracks and switch off the fan.

After removing the lid, the wick is lit with a lighter or match. The room must be vacated immediately after lighting. Within 60 seconds, the active substance is expelled. The generator

produces a greyish white smoke without a flame. For safety reasons, the smoke generator should be placed on a non-flammable surface (brick, tile...) as the container itself may become very hot and remain so for at least 5 minutes.

The smoke should be allowed to settle and contact surfaces for no less than 30 minutes but ideally 12 hours. Early entry should be permitted only in case of emergency or to operate ventilation equipment. Personal protective equipment is required.

Safe when used according to directions

Avoid inhalation of the smoke because of the possible irritating effect of the smoke itself. Treated areas should not be re-entered for at least 2 hours after treatment.

Clinafarm® Smoke Generators have a shelf life of three years at room temperature (< 86°F).



3.6. Control to prevent infection rather than cure

Reducing infection pressure at all levels

When controlling aspergillosis in broiler chicks, good management at all levels of the production chain are essential. Infection pressure should be reduced to a minimum by proper risk management at all production levels, including breeder and broiler farms.

At breeder farm level, risk factors include:

- poor shell quality due to poor feed quality, disease or aging flocks
- excessive shell breakage and egg sweating due to poor temperature management
- dirty eggs due to poor nest management or collection practices
- inadequate storage or transport

At hatchery level, the risk factors include:

- contaminated, cracked or dirty eggs coming in from the breeding farm
- contaminated or insufficiently cleaned fans, filters, equipment, etc.
- badly adjusted transfer machines (damaged eggs, source of contamination)
- poor management of *in ovo* vaccination procedures

- badly adjusted chick separators/counters (dust and stress)
- contaminated cardboard or wooden material
- poor biosecurity (insufficient cleaning, open doors...)
- poor hatchery design: no separation between 'dirty' and 'clean' areas, the use of disinfectants with limited anti-fungal activity

At broiler farm level, the main risk factors are:

- poor quality or wet shavings, hardwood litter, dusty litter
- contaminated feed due to feed remnants left behind in the bins (cross-contamination)

Control programs

Clinafarm® is of particular interest when used preventively. Broiler producers will note that chicks are of a better quality and are livelier, and that flocks are more uniform.

An aspergillosis control program should involve the use of Clinafarm® EC and Clinafarm® Smoke Generator in the standard hatchery cleaning and disinfection procedures, including the regular disinfection of the ventilation ducts in the hatchery with Clinafarm® Smoke Generators. Use Clinafarm® Smoke Generators to disinfect empty egg rooms or egg delivery trucks to reduce the incidence of *Aspergillus* entry to the hatchery.



Poor quality hatching eggs can be a source of *aspergillus* entry into the hatchery. Eliminate these eggs prior to delivery.

4.1 Hatcheries

All areas of the hatchery should be thoroughly cleaned but particular areas will certainly require extra attention. As the spores need organic material as a growth substrate, the egg storage, hatching and chick handling rooms as well as the waste areas are the main sources of contamination. Regular sampling should give an idea of the main *Aspergillus* proliferation sites.



Clinafarm® treatment in hatcheries		
Area to disinfect	When?	Clinafarm®
Egg room	Before eggs arrive	EC at 1: 100 spray / fog or 1 Smoke Generator per 400 – 500 ft ³
Incubators	When emptied for cleaning	EC at 1: 100 or 1 Smoke Generator per 400 – 500 ft ³
Hatchers	When emptied for cleaning	EC at 1: 100 or 1 Smoke Generator per 400 – 500 ft ³
Refrigeration units, humidifiers		EC at 1:100 spray
Chick processing area, empty trays, equipment	Spray or fog after use	EC at 1:100 spray or fog
Other rooms	2 x per week	EC at 1:100 spray or fog
Ventilation systems, filters, exhausts	Clean as well as possible	1 Smoke Generator per 400 – 500 ft ³
Evaporative cooling systems	2 x per week	EC at 1:100 spray
Air-conditioning units	2 x per week	EC at 1:100 spray

Remarks

- considered a 'dirty' area
- avoid pressure hoses
- remove any cardboard trays
- avoid using in the presence of eggs

- keep multi-stage setters as clean as possible
- remove cracked eggs

- major area of contamination
- clean thoroughly prior to spraying

- mix diluted Clinafarm® EC liquid in humidifier bowls

- Careful attention to:
- filter maintenance
 - coil cleaning (especially inside coil)
 - directional grill
 - condensation pans



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Label information

Clinafarm® EC (Emulsifiable Concentrate)

Clinafarm® Smoke Generator
Brand of Imazalil (enilconazole*)

Veterinary Use Only

Composition

Clinafarm® EC contains Imazalil (enilconazole): 1-(2-(2,4-Dichlorophenyl)-2-(2-propenyloxy ethyl)-1H-imidazole (13.8% ww) per bottle; Clinafarm® Smoke Generator contains Imazalil (enilconazole): 1-(2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl)-1H-imidazole (14.9% ww) per canister.

Properties

Clinafarm® EC or Clinafarm® Smoke Generator contains imazalil, a fungicide. Clinafarm® can be used for the disinfection of poultry and turkey hatchery equipment prior to the introduction of eggs. Major causes of infections, sensitive to imazalil, are *Aspergillus* organisms.

Directions for Use

Clinafarm® EC: For disinfection of hatchery equipment. Equipment would include empty hatchery cabinets, setters, coolers, storerooms, handling equipment, etc. Clinafarm® EC will dramatically reduce the levels of infectious organisms and spores in treated areas. To make 1 gallon of 1:100 dilution, add 1.25 fl. oz. (36 mL) of Clinafarm® EC to one gallon of clean water to provide an antifungal concentration of 0.15% active ingredient. Fog or spray area to be treated at the rate of 1/2 fl. oz. diluted product per 150 cu. ft. (1 mL per 10 cu. ft.). Fog or spray to ensure good contact with surface areas. Do not re-enter treatment areas for two hours. Clinafarm® EC is compatible with other commonly used disinfectants such as quaternary ammonium compounds, glutaraldehyde, formaldehyde, and phenolic compounds.

Clinafarm® Smoke Generator: Do not remove canisters from container except for immediate use. Treatment of the hatchery equipment should be the final step prior to introduction of the eggs. After the equipment has been thoroughly cleaned and disinfected, a smoke canister of imazalil should be placed on a noncombustible surface. Before starting, the area to be treated should be closed off to prevent air currents from diluting smoke. Ventilation ducts may be treated, but fans must be shut off. The wick of the canister should be ignited and all persons should leave the treated area. Within 60 seconds the canister will be through smoking. The smoke should be allowed to settle and contact the surfaces for no less than 1/2 hour but preferably up to 12 hours before fumigated equipment and areas are used. Delay reentry into treatment areas until the smoke has settled or dissipated to avoid possible eye irritation. Do not reenter unventilated areas for 12 hours after treatment. For ventilated areas, do not reenter treatment areas until ventilation ducts and fans have been opened and on for at least 30 minutes and at least 1-2 air exchanges have occurred. For either unventilated or ventilated areas. Do not reenter if treatment smoke is still visible.

Precautionary statements

Hazards to humans and domestic animals.
Keep out of reach of children.
See labels for additional First Aid and Precautionary Statements.

How supplied

Clinafarm® EC: 4 x 750 mL tip "N" measure® bottles/case
Clinafarm® Smoke Generator: 24 canisters/case

NOTE: See Clinafarm® EC and Clinafarm® Smoke Generator labels for complete directions and First Aid and Precautionary Statements.

* Tradename is not approved in US.

Mold sample sheet

Hatchery: _____ Recorded by: _____ Date and time of sampling: _____

Read by: _____ Date and time of reading: _____

Mold samples collected on Sabourard-dextrose media. Ten minute air plates unless swabs are used. Report number of various types of mold colonies for each sample.

Note: All hatcher air plates must be done with the hatchers being clean, sanitized, dry, up to temperature and with no eggs present.

AFTER SAMPLING, ALL PETRI DISH LIDS MUST BE TAPED CLOSED.

SAMPLE NUMBER	LOCATION	Aspergillus SP	other blue/ green mold	non blue/ green mold	comments
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

Conclusions

Aspergillosis is a worldwide problem in all the different stages of the poultry and game bird production chain. Hatcheries are particularly at risk due to the favorable growing conditions for the mold. The main source of infection is contaminated eggs from the breeder farm.

Contaminated hatcheries will observe a drop in hatchability and early mortality, while surviving chicks will show a poor performance later in life—at the broiler farm. Infected animals cannot be treated while contaminated hatcheries can never be decontaminated completely. Good Clinafarm® control programs at the hatchery are therefore essential.

Clinafarm® disinfectants are highly effective against both fungi and spores and offers the poultry industry a unique and versatile tool to prevent and overcome aspergillosis.



Hatchability and Egg Necropsy Record individual trays

How to use this form?

This form was designed to structure and compare different egg necropsies that are performed on a regular basis in every hatchery. It provides the means to come to a correct insight of the hatchery performance and allows perfect problem identification and quantification.

Hatchery: _____ Recorded by: _____ Treatment description: _____ Date: _____

Flock Name: _____ Flock Age: _____ Egg Age: _____ Setter number: _____ Hatcher number: _____

Age at transfer: _____ Number of eggs set: _____

number of tray	1	2	3	4	5	TOTALS
1. Early mortality & non viable eggs						
infertiles						
early dead						
middle dead						
rot						

Infertile egg: A non fertilized egg. Normally a bright yolk, looking like a fresh egg, no sign of blastoderm development.

Early dead: Dead between 0-7 days of incubation. Signs of blastoderm development, followed by blood ring 48-72 hrs, egg tooth appears at day 7.

Middle dead: Dead between 8-14 days (note: normally, there should be no deaths occurring between 8-14 days). Appearance of easily visible embryonic eye vesicula, egg tooth, visible yolk stalk. No feather follicles covering the ear hole.

Rot: An infected egg, may smell.

number of tray	1	2	3	4	5	TOTALS
2. Anomalies						
malformed						
malpositioned						
total 2. (A)						

Malformed: Any physical deformity, i.e. brain exposed, extra legs, deformed beak, eyes missing, dwarf, etc.

Malposition: Chick upside down, legs over head, head under left wing etc.

number of tray	1	2	3	4	5	TOTALS
3. Full Term Embryos Remaining After Hatch						
late dead						
live pip						
dead pip						
live not pipped						
cull/dead						
total 3. (B)						
total A+B						

Full term embryos remaining after hatch: All embryos in this category will have feather follicles covering the ear and the yolk stalk will have been absorbed into the abdomen.

Late dead: Dead between 14-21 days. Normally formed, fully developed, dead embryo which has not pipped the shell, head under right wing.

Live pip: Normally developed embryo, has pipped the shell and is alive.

Dead pip: Normally developed embryo, has pipped the shell but dead.

Live not pipped: Normally formed embryo which is still alive but has not started to pip the shell.

Cull/dead: A dead or 2nd grade chick found in the hatcher basket.

number of tray	1	2	3	4	5	TOTALS
4. Normal hatched chick / poult counted						
total 4. (C)						
total A+B+C						

number of tray	1	2	3	4	5	TOTALS
5. Non exclusive categories						
cracked (incubation)						
cracked (inj / transfer)						
visible mold						

Intervet/Schering-Plough Animal Health

56 Livingston Avenue
Roseland, New Jersey 07068

Technical Service: 1-800-211-3573
Customer Service: 1-800-356-7470



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