

USE OF CHLORINE DIOXIDE IN HATCHERY

*SAFETY AND EFFICACY AGAINST ASPERGILLUS
INFECTIONS*

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Chlorine dioxide

greenish yellow gas at room temperature.

- water soluble, solutions quite stable
- extremely reactive compound, cannot be manufactured and shipped in bulk, it is prepared in place of consumption
- typically this involves complicated machinery and handling of hazardous chemicals. These systems are appropriately designed for large industrial users but are not appropriate for small use consumers.

Used in number of industries:

- pulp bleaching
- water treatment
- processing plants
- fruit and vegetables treatment in effort to control spoilage

- *ICA Z-Series 2000-G* impregnates precursor chemicals, e.g. acids, chlorite or chlorate salts into porous particles. Combining these particles stimulates surface chemistry that releases chlorine dioxide gas.
- *2000-G* media is designed to release controlled amounts of Chlorine Dioxide gas over time
- *2000-G* medias are packaged in gas permeable sachets and labeled for reliable production
- *2000-G* medias are non-flammable, stable and environmentally friendly
- *2000-G* solid media is designed for safe and convenient Chlorine Dioxide generation
- *2000-G* products are designed to produce milligrams to grams of Chlorine Dioxide over fixed time intervals to match user needs
- After full use of *2000-G* sachet, discard as regular non-hazardous waste

Three test incubators at PDRC, automatic turning mechanism, controlled temperature and humidity



Four test groups, 210 eggs each, divided in three subgroups of 70 eggs



EXPERIMENT 1

Incubator 1 – no ClO₂

Incubator 2 –4.3 ppmv AVG ClO₂/day from day 1 till end

Incubator 3 - ClO₂ continuously from day 18 (when eggs were transferred to hatching baskets) – 61 ppmv ClO₂/day for the first day, 2.1 ppmv for the second and 0.7 ppmv for third day

Four groups:

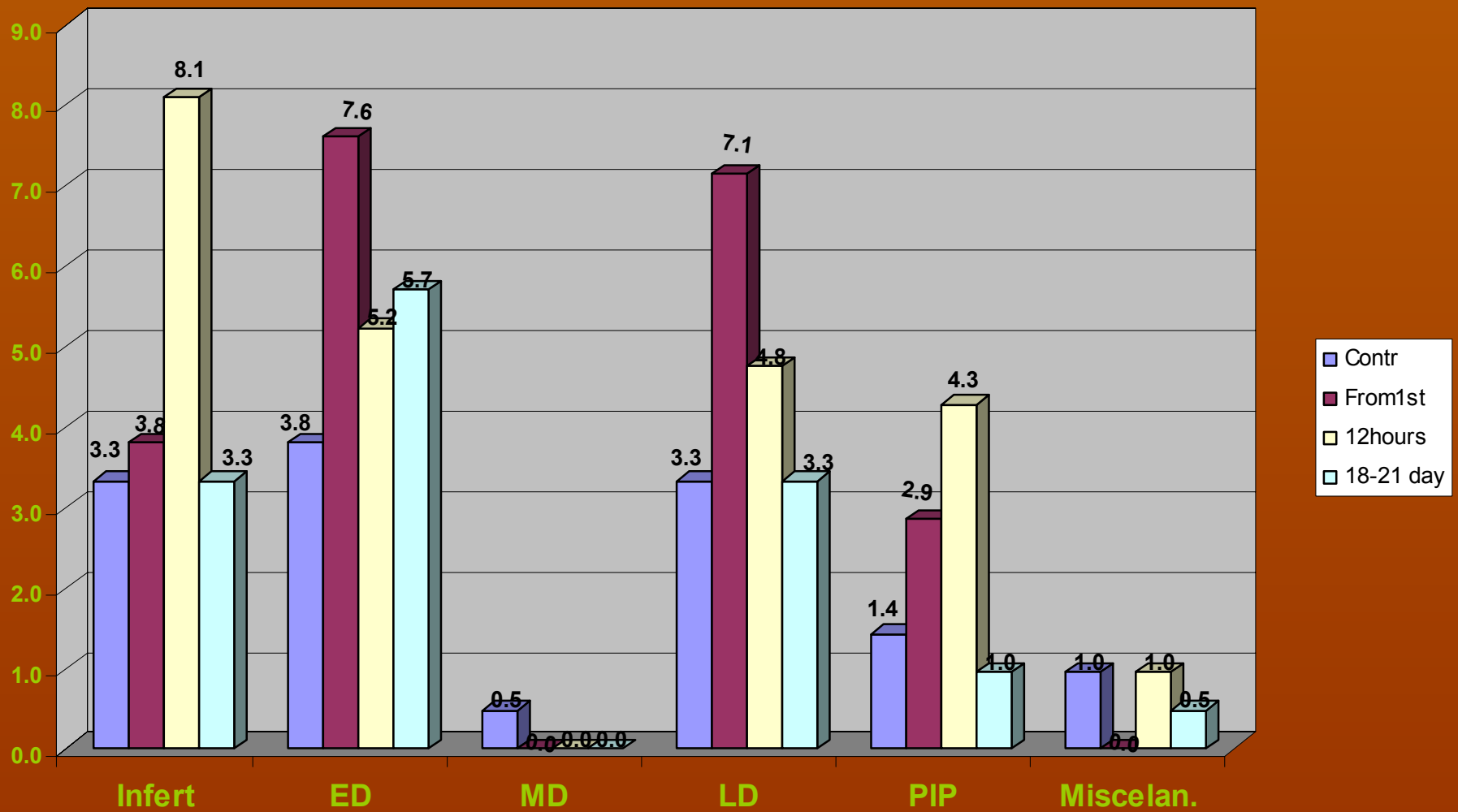
#1 – control eggs, no treatment

#2 – treated with ClO₂ from the first day till hatching

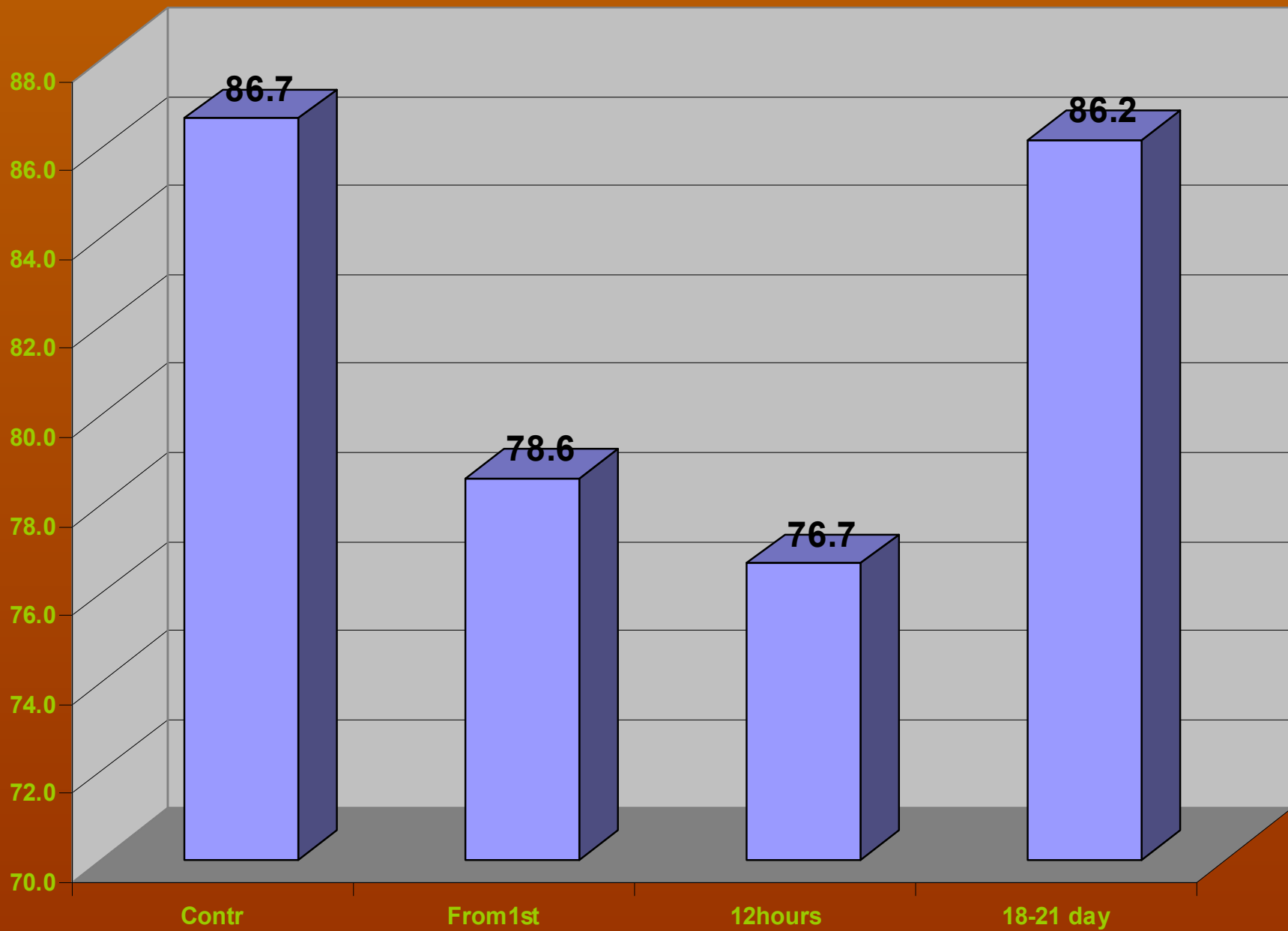
#3 – eggs treated with ClO₂ for 12 hours on days 1,4,7,10,13,16 (moved to incubator 2) and continuously from day 18-21

#4 – treated with ClO₂ from day 18-21

Treatment	Infertile	ED	Inf+ED	MD	LD	PIP	Hatchability	Miscellaneous	Hatch fertile	of
Control	3.4 ^A	3.8 ^A	7.1 ^A	0.5 ^A	3.3 ^A	1.4 ^A	86.7 ^A	0.9 ^A	89.7 ^A	
From 1 st	3.8 ^A	7.6 ^B	11.3 ^{AB}	0 ^A	7.1 ^B	2.9 ^{AB}	78.6 ^B	0 ^A	81.7 ^B	
12 hours	8.1 ^B	5.2 ^{AB}	13.3 ^B	0 ^A	4.8 ^{AB}	4.3 ^B	76.6 ^B	0.9 ^A	83.4 ^B	
18-21 day	3.3 ^A	5.7 ^{AB}	9.1 ^{AB}	0 ^A	3.6 ^A	1.0 ^A	86.2 ^A	0.5 ^A	89.1 ^A	



% of Hatchability



EXPERIMENT 2

Incubator 1 – no ClO₂

Incubator 2 –4.3 ppmv aver. ClO₂/day from day 1 till end

Incubator 3 – bags for 12 hours treatment (same concentration) every 3 days + continuous treatment 18-21 day (61 ppmv ClO₂/day for the first day, 2.1 ppmv for the second and 0.7 ppmv for third day)

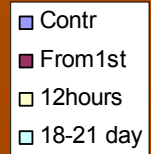
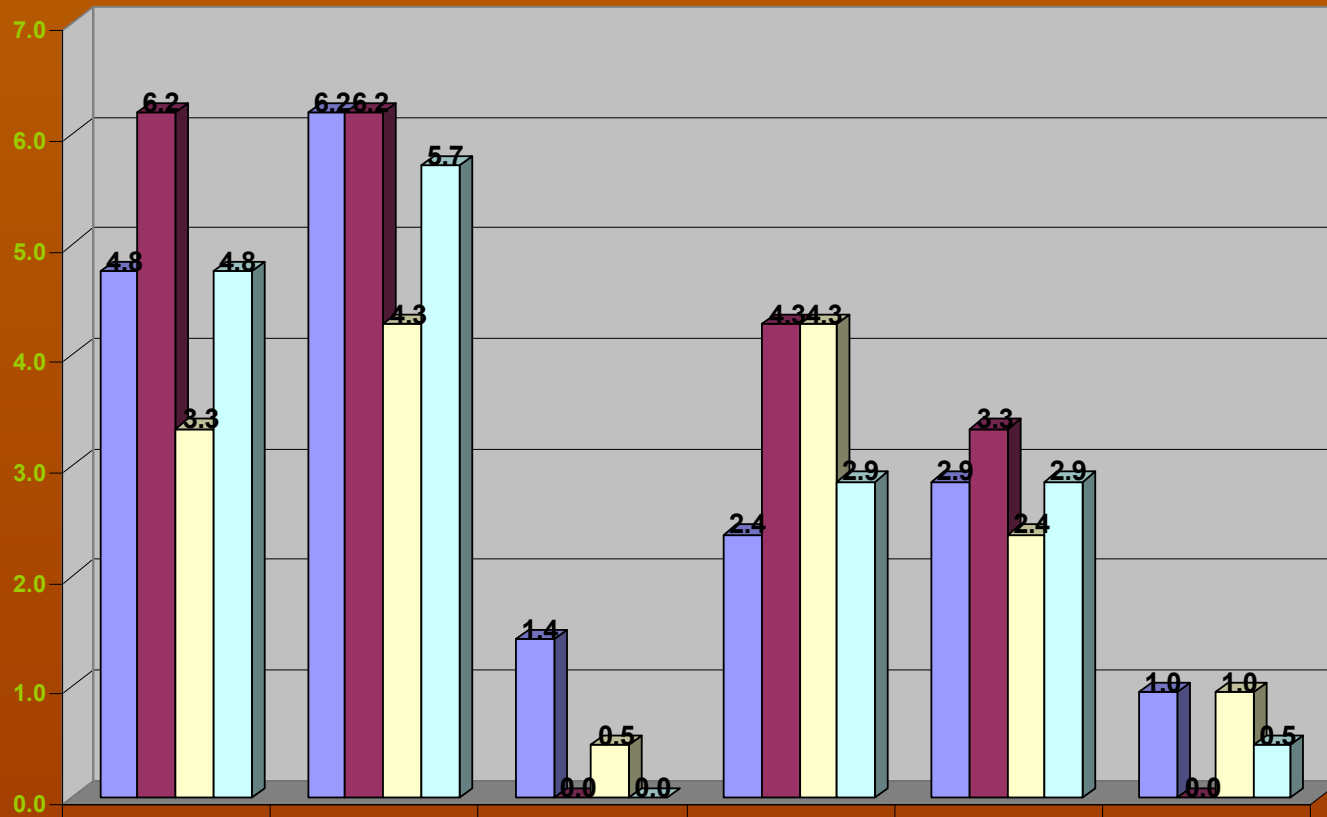
Four groups:

#1 – control eggs, no treatment

#2 – treated with ClO₂ from the first day till hatching

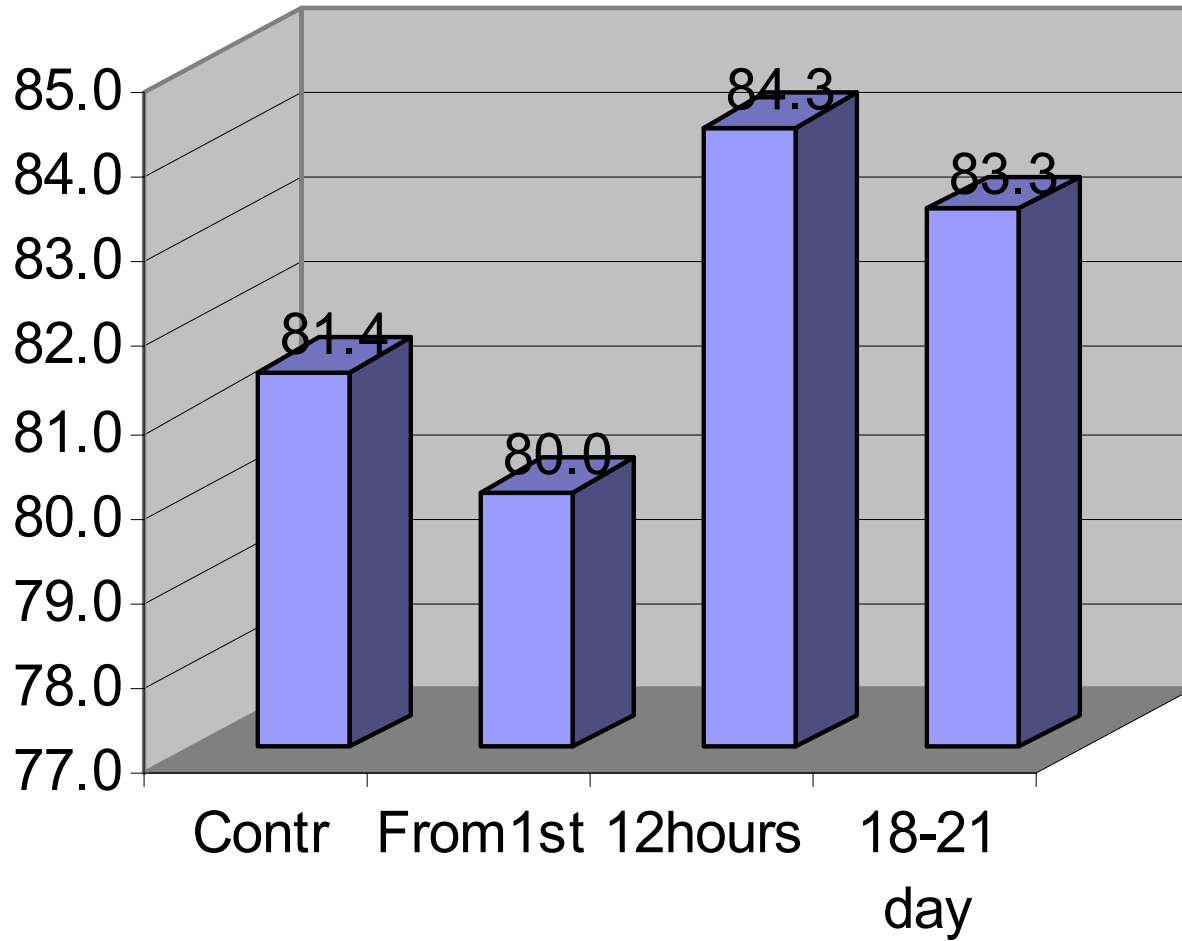
#3 – eggs treated with ClO₂ for 12 hours on days 1,4,7,10,13,16 and continuously from day 18-21

#4 – treated with ClO₂ from day 18-21



	Infert	ED	MD	LD	PIP	Miscelan.
Contr	4.8	6.2	1.4	2.4	2.9	1.0
From1st	6.2	6.2	0.0	4.3	3.3	0.0
12hours	3.3	4.3	0.5	4.3	2.4	1.0
18-21 day	4.8	5.7	0.0	2.9	2.9	0.5

% of hatchability



EXPERIMENT 3

Incubator 1 – no ClO₂

Incubator 2 – 21.6!! ppmv aver. ClO₂/day from day 1 till end

Incubator 3 – around 20 ppm of formaldehyde from day 1 till end

EGGS CANDELED ON DAY 8 AND 12 - IN ClO₂ TREATED GROUP ALL BUT 2 EGGS WERE DEAD (9 OF 210 WERE INFERTILE)

EFFICASY OF ClO_2 AGAINST ASPERGILLUS FUMIGATUS

- Egg contamination procedure:
- About 5 ml 0.5% TRITON X-100 put into a plate with Aspergillus
- Washed out and filtered through 0.2 μm filter. Procedure repeated three times until all spores from a plate were washed out.
- The filtrate suspended in about 5l 0.005% TRITON solution of 40 °C
- Eggs dipped into the solution for about 30 seconds and dried



Three groups of Not contaminated (“Clean”) eggs - #1 not treated, #2 treated with low level of ClO_2 and #3 treated with high level of ClO_2

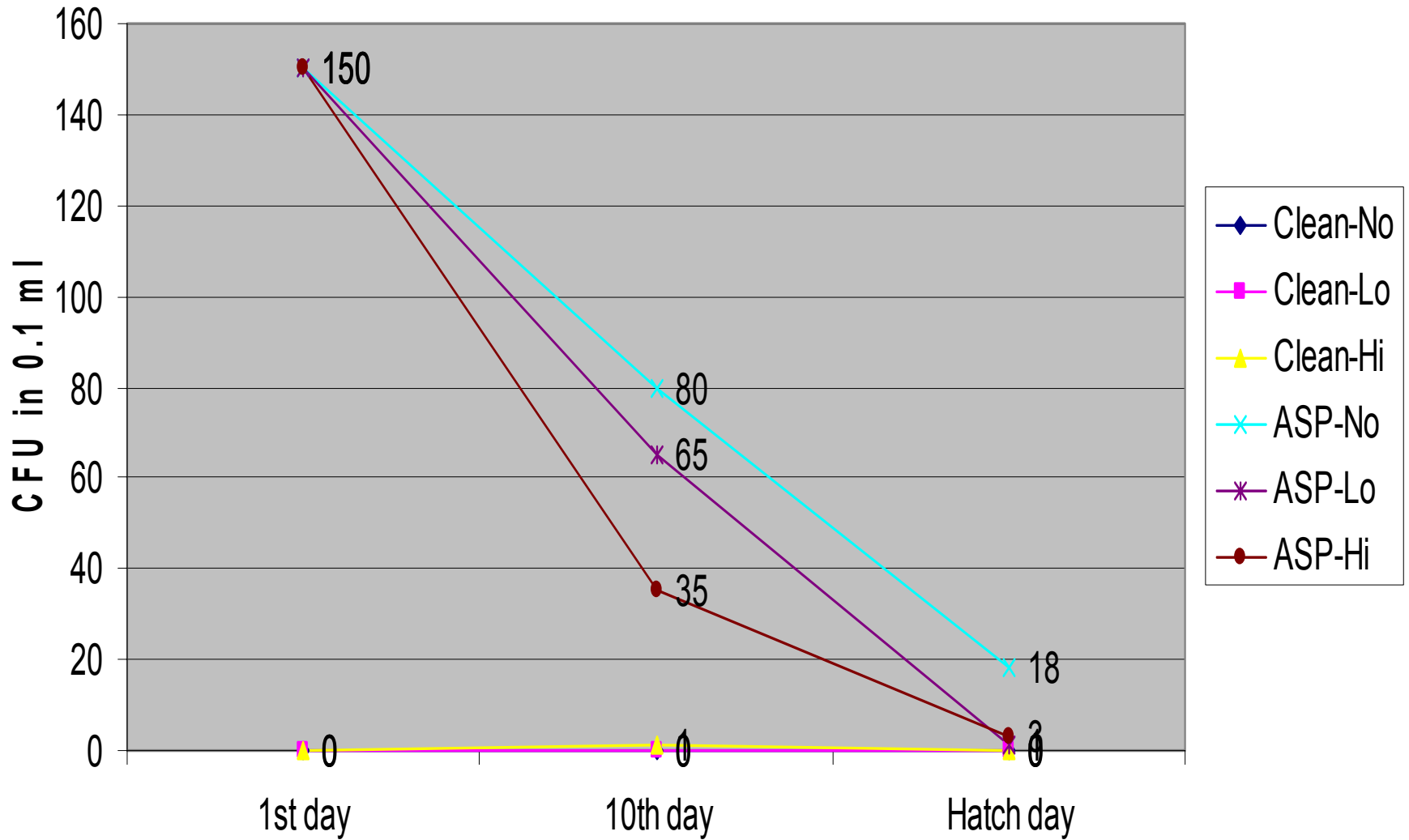
Three groups of contaminated eggs - #1 not treated; #2 treated with low level of ClO_2 and #3 treated with high level of ClO_2



- Five eggs per group washed separately in 50 ml of washing solution (0.05% Triton) for 3+3+3 minutes
- 10^{-1} and 10^{-2} dilutions made
- 0.1 ml of original wash and of dilutions transferred to SDA plate, run in duplicates
- Number of colonies (CFU) counted after 24 hours at 37 C and 1-2 days at room temperature



Egg-wash



CONCLUSIONS

- Generally, embryos are negatively affected by early continuous exposure to ClO₂. The adverse effect decreases at later stages of development of embryo.
- Treatment of hatching eggs in the hatcher (18-21 day) proven to be safe
- Short (12 hour) treatment of eggs in the setter, every time a new batch of eggs is moved in, seems to be also safe
- Treatment with the levels of ClO₂ safe for eggs seems to be effective in control of Aspergillus contamination
- Repeatable and accurate challenge model is needed



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