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(54) Title: NEW DISINFECTANT FOR HATCHERIES

(57) **Abrégé/Abstract:**

The invention relates to a stable disinfectant composition and the use of said composition for spraying hatching eggs prior to in ovo vaccination, for disinfecting vaccination equipment including vaccination needles, and for flushing vaccination equipment post vaccination cycles.

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NEW DISINFECTANT FOR HATCHERIES**FIELD OF THE INVENTION**

The invention relates to a stable disinfectant composition and the use of said composition
10 for spraying hatching eggs prior to *in ovo* vaccination, for disinfecting vaccination equipment
including vaccination needles, and for flushing vaccination equipment post vaccination
cycles.

15 BACKGROUND OF THE INVENTION

In ovo injection has become an important tool to administer vaccines in the hatcheries.
Nevertheless, some basic pre-cautions must be taken into account in order to achieve the
best results with this technology. Good sanitation of the hatchery, thorough
20 disinfection/decontamination of the hatching eggs are among these special
cares. Furthermore, precise maintenance of the machine is compulsory by way of preventing
biofilm generation. By ensuring that these procedures are properly followed, the industry
can ensure benefit from this interesting and powerful *in ovo* vaccination technology.

25 Chlorine releasing tablets, powders and solutions are the standard disinfection methods in
the modern hatchery industry. However, the chlorine release method has the main
disadvantage that chlorine is corrosive to several metals and alloys encountered in the
hatchery sector and in *in ovo* vaccination equipment. Moreover, effectiveness of chlorine
disinfectants against some microbial pathogens is weakened when organic matter is present.

30

Hydrogen peroxide (H_2O_2) is also commonly used at a concentration of 3% weight/volume.
 H_2O_2 is known to release highly oxidizing nascent oxygen {O} which has strong corrosive
action on metal and alloy surfaces apart from being a disinfectant. Handling of stock

5 solutions of H₂O₂ is also considered to be risky for the end user on account of the high reactivity of H₂O₂ with human skin.

Disinfectant compositions of the prior art cannot be efficiently used in industrial hatcheries because they are too corrosive for the high tech *in ovo* vaccination equipment, because they
10 are not environmental friendly, because disinfectant compositions are often unstable, because they often cause user safety issues, and because of the limited speed of action against pathogens. Indeed, conventional *in ovo* vaccination requires killing of the key pathogens found in hatcheries (*E. coli*, *P. aeruginosa*, *Salmonella spp*, and *Staphylococcus aureus*) within 3 seconds of exposure which is the average time between two *in ovo* injection
15 cycles.

Therefore, there is still a need in the industry for a disinfectant composition which is easy to prepare at room temperature prior use, which is free of halogenated chemical compounds, free of aldehydes, which is stable for at least two years in powder or tablet form, which can
20 be solubilized in any tap water within minutes at room temperature, which is stable for at least 6 hours in liquid form, while providing a complete disinfection within three seconds of contact with microbial pathogens. Moreover, said disinfectant composition should not lead to any corrosion of metallic parts of the equipment being sanitized/disinfected, nor provoke any dis-coloration of the non-metallic parts of the equipment. Furthermore, there is also a
25 need for a composition which, when used as a liquid disinfectant has a broad spectrum of antimicrobial efficacy against the main pathogens found in hatcheries and is safe for human handling.

30 SUMMARY OF THE INVENTION

Surprisingly, the inventors found that the composition of the present invention overcomes the foregoing problems by providing a stable composition in solid and liquid form, a composition free of halogenated chemical compounds, a composition easily solubilized in
35 tap water and at room temperature, and a composition being effective within 3 seconds

5 while retaining efficacy in a broad range of tap water comprising various salts, being safe to the user, and not leading to any corrosion nor discoloration upon repeated use.

In a first aspect, the aim of the present invention is to provide a stable solid disinfectant composition. A further aspect of the invention is to provide the use of this stable solid
10 composition, after rapid solubilization in tap water, to disinfect eggs in hatcheries prior *in ovo* vaccination as well as vaccination equipment (injection needles) between every two consecutive *in ovo* injections during vaccination cycles.

A further object of the present invention is to provide a method of disinfecting eggs and
15 hatchery equipment, including vaccination equipment, comprising the steps of dissolving a stable disinfectant composition in tap water at a concentration of 1 to 5%, and contacting the disinfectant solution for 3 to 5 seconds with surfaces to be treated.

DETAILED DESCRIPTION OF THE INVENTION

20

In a first aspect, this invention relates, to a stable disinfectant composition comprising:

- a. 40 to 50 wt % sodium percarbonate
- b. 20 to 25 wt % tetra acetyl ethylene diamine
- c. 10 to 20 wt % anhydrous citric acid
- 25 d. 0 to 2 wt % alpha olefin sodium sulfonate
- e. 5 to 15 wt % anhydrous sodium acetate
- f. 0.01 to 0.02 wt % of an anti-corrosive agent
- g. 5 to 20 wt % of a filler.

30 Disinfectants are antimicrobial agents that are applied to the surface of non-living objects to destroy microorganisms that are living on the objects.

As used herein, the term "wt %" means a weight/weight ratio relative to the total weight of the composition.

35

5 Sodium percarbonate (CAS 15630-89-4) or peroxy sodium carbonate, or sodium carbonate peroxide, or sodium carbonate peroxyhydrate is an adduct of sodium carbonate and hydrogen peroxide (a perhydrate), with the following formula ($2 \text{ Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}_2$). It is a colorless, crystalline, hygroscopic and water-soluble solid. Sodium percarbonate is more eco-friendly than sodium perborate commonly used in the industry because it
10 degrades into oxygen, water and sodium carbonate.

Tetra acetyl ethylene diamine (TAED) (CAS 10543-57-4) is a peracetic acid generator. A suitable supplier of TAED is Warwick Chemicals. The TAED may be an uncoated TAED, for example a uncoated powdered form of TAED. A suitable TAED is MykonR B675 TAED.

15 Alpha olefin sodium sulfonate or sodium C_{14-16} alpha olefin sulfonate surfactants provide outstanding detergent activity with a high compatibility with hard water, and good wetting and foaming properties. It comprises approximately 90 to 95% sulfonic acids, C_{14-16} -alkane hydroxy and C_{14-16} -alkene, sodium salts with the remaining 5 to 10 % Sodium Sulfate. They
20 are available from Parchem.

Anhydrous citric acid is used in the present composition as a pH controller and buffer agent. Anhydrous means that the substance has no or limited amount of water and is in a dry, granulated form.

25 Anhydrous sodium acetate also abbreviated NaOAc also known as sodium ethanoate, is the sodium salt of acetic acid.

Anti-corrosive agent or corrosion inhibitor is a chemical compound that, when added to a liquid, decreases the corrosion rate of a material, typically a metal or an alloy. Any liquid
30 anti-corrosive agent may be used in the composition of the present invention. Preferred anti-corrosive agent is sodium benzoate.

For the composition according to the present invention, any filler which is highly soluble in water may be used. Preferred filler according to the present invention is the inorganic
35 compound with formula Na_2SO_4 : sodium sulfate.

5

The composition according to the present invention preferably comprises:

- a. 42 to 48 wt % sodium percarbonate
- b. 22 to 23 wt % tetra acetyl ethylene diamine
- c. 12 to 18 wt % anhydrous citric acid
- 10 d. 0 to 2 wt % alpha olefin sodium sulfonate
- e. 8 to 11 wt % anhydrous sodium acetate
- f. 0.01 to 0.02 wt % of an anti-corrosive agent
- g. 5 to 11 wt % of a filler.

15 More preferably, the composition according to the present invention comprises:

- a. 44 to 46 wt % sodium percarbonate
- b. 22 to 23 wt % tetra acetyl ethylene diamine
- c. 14 to 16 wt % anhydrous citric acid
- d. 0 wt % alpha olefin sodium sulfonate
- 20 e. 9 to 10 wt % anhydrous sodium acetate
- f. 0.01 to 0.02 wt % of sodium benzoate
- g. 5 to 8 wt % of sodium sulfate.

In a preferred embodiment, the composition according to the present invention is provided
25 in a solid form including free flowing powder form or tablet. Preferably the composition is in a powder form, and packaged in bags, sachets or pouches. Even more preferably, sachets or pouches are made of water soluble material such as polyvinylalcohol as it is well known to the person skilled in the art.

30 Prior using the composition as a liquid disinfectant, the composition needs to be dissolved in tap water. Effective concentration of the composition according to the present invention is reached when the composition is dissolved at 1 to 5 % concentration in water at room temperature, preferably 2 to 3 %. Therefore, for the ease of use, and for safety purposes, unidose packages are provided for predefined amounts of water.

35

5 Surprisingly, the present composition when used in powder form was found to dissolve very rapidly in tap water at room temperature within 5 minutes and at very different concentrations of Mg, Ca or Na salts.

Another object of the present invention is to provide the use of a composition comprising:

- 10 a. 40 to 50 wt % sodium percarbonate
b. 20 to 25 wt % tetra acetyl ethylene diamine
c. 10 to 20 wt % anhydrous citric acid
d. 0 to 2 wt % alpha olefin sodium sulfonate
e. 5 to 15 wt % anhydrous sodium acetate
15 f. 0.01 to 0.02 wt % of an anti-corrosive agent
g. 5 to 20 wt % of a filler,

wherein said composition is dissolved in tap water at room temperature at a concentration of 1 to 5 % as a disinfectant for spraying eggs prior embryo vaccination, for spraying egg vaccination equipment, and/or for flushing of vaccination equipment. Preferably, the
20 composition is dissolved at a concentration of 2 to 3 %.

In ovo vaccination is carried out by machines. These machines perform a number of actions to ensure good vaccination of the chick inside the egg. Benefits of *in ovo* vaccination include uniformity in mass vaccination, avoidance of bird stress, controlled hygienic conditions, and
25 earlier immunity with less interference from maternal antibodies. However, the entire *in ovo* vaccination process should be performed in sterile condition so as to prevent any introduction of microbial pathogens and/or contaminants into the eggs.

In a preferred embodiment, the present composition dissolved in water is effective in killing
30 *E. coli*, *P. aeruginosa*, *Staphylococcus spp*, *Salmonella, spp*, such as *S. gallinarum*, or *S. enteritidis*.

Another object of the present invention is a method of disinfecting eggs and hatchery equipment, including vaccination equipment, comprising the steps of dissolving a
35 composition comprising:

- 5 a. 40 to 50 wt % sodium percarbonate
- b. 20 to 25 wt % tetra acetyl ethylene diamine
- c. 10 to 20 wt % anhydrous citric acid
- d. 0 to 2 wt % alpha olefin sodium sulfonate
- e. 5 to 15 wt % anhydrous sodium acetate
- 10 f. 0.01 to 0.02 wt % of an anti-corrosive agent
- g. 5 to 20 wt % of a filler,

in tap water at room temperature and at a concentration of 1 to 5%, and contacting the disinfectant solution for 3 to 5 seconds with surfaces to be treated.

- 15 All embodiments described above for the stable disinfectant composition also apply to the use of said composition, and to the method of disinfection as described above.

5 **EXAMPLES**Example 1: Stable Disinfectant Composition

	sodium percarbonate	45 wt %
10	tetra acetyl ethylene diamine	22.5 wt %
	anhydrous citric acid	15 wt %
	alpha olefin sodium sulfonate	1.44 wt %
	sodium benzoate	0.016 wt %
	anhydrous sodium acetate	9.5 wt %
15	sodium sulfate	6.55 wt %

Example 2: Stable Disinfectant Composition

	sodium percarbonate	44.5 wt %
20	tetra acetyl ethylene diamine	23 wt %
	anhydrous citric acid	15 wt %
	alpha olefin sodium sulfonate	1.44 wt %
	sodium benzoate	0.016 wt %
	anhydrous sodium acetate	9.5 wt %
25	sodium sulfate	6.55 wt %

Example 3: Stable Disinfectant Composition

	sodium percarbonate	45.5 wt %
30	tetra acetyl ethylene diamine	22 wt %
	anhydrous citric acid	15 wt %
	alpha olefin sodium sulfonate	1.44 wt %
	sodium benzoate	0.016 wt %
	anhydrous sodium acetate	9.5 wt %
35	sodium sulfate	6.55 wt %

5 Example 4: Dissolution rate of the product of Example 1 compared to Perasafe

Perasafe is a composition of the prior art comprising 50 wt % perboric acid sodium salt, 1 wt % sodium carbonate and 15 wt % citric acid

10 Both compositions were mixed at 2 % concentration in distilled water, and dissolution visually observed after 5 minutes stirring at 187 rpm. Table 1 shows that the composition according to Example 1 dissolves more efficiently than the disinfectant of the prior art Perasafe.

Formulation	Concentration in distilled water	Temperature of water	Rotation speed (rpm)	Observation after 5 mins.	Clarity of solution
Composition of Example 1	2 %	25°C	187	Completely dissolved	Clear
PERASAFE	2%	25°C	187	Small quantity remains undissolved	Clear

15

Table 1: Comparative dissolution.

Example 5: Antimicrobial efficacy assays

20 Antimicrobial efficacy assays were performed with compositions according to Example 1 at 2, 3, and 4 % dilution in water.

Material and methods:

Equipment: Laminar air flow. (ENVAIR), Stop watch, Cyclo mixer (REMI CM 101DX), and
25 Incubator operated at 37°C (LABTOP)

Chemicals: Sodium meta bisulphite (A.R.) solution (100 mg/ml), Nutrient broth (HIMEDIA)

Tested microorganisms: *Ps. Aeruginosa* (ATCC9027), *E. coli* (ATCC 8139), *S. abony*(NCTC 6017), suspensions having count 10^6 cfu/ml.

5 Preparation of a 2 % liquid solution of the composition of Example 1: Weight accurately 2 g of the composition of Example 1 and dissolve in 100 ml distilled water. Stir sample continuously for about 5 minutes at room temperature until it dissolves completely to obtain a clear solution.

10 Bactericidal assays: Bactericidal assay were carried out as detailed below.

1 Positive Control

To 8 ml nutrient broth add 100 μ l of *P. aeruginosa* suspension (10^6 cfu/ml) and mix well. Incubate at 37°C for 48 hrs.

15 After completion of incubation period observe solution for turbidity (bacterial growth).

2 Negative Control

Incubate 8 ml nutrient broth at 37°C for 48 hrs. After completion of incubation observe solution for turbidity (bacterial growth).

20

3 Test

To 8 ml 2% composition according to Example 1 solution add 100 μ l of *P. aeruginosa* suspension (10^6 cfu/ml) with continuous stirring using cyclo mixer and expose for 3 seconds. Add 50 μ l of neutralizer (100 mg/ml) immediately at the end of 3 seconds to the solution stirring continuously and allow standing for 30 seconds (solution A).

25

Then transfer 1 ml of solution A to the tube containing 9 ml nutrient broth, mix well and incubate at 37°C for 48 hrs.

After completion of incubation period observe the solutions for turbidity (bacterial growth).

Repeat same procedure for 3 % and 4 % solution with the composition of Example 1 as well

30 as with the other microbial strains *E. coli* and *S. abony*. Results are shown in Table 2:

Pathogens at 1×10^6 cfu/ml	Contact time	% killing efficacy with a solution prepared from composition of Example 1 dissolved in water at:		
		2%	3%	4 %
<i>E. coli</i>	3 seconds	100	100	100

<i>P. aeruginosa</i>	3 seconds	100	100	100
<i>S. aureus</i>	3 seconds	100	100	100
<i>S. abony</i>	3 seconds	100	100	100

5

Table 2: Killing efficacy results

It may be seen from the above results that an exposure for 3 seconds to 2 %, 3% and 4 % of the composition according to Example 1 in solution exhibits complete killing of *E. coli*, *P. aeruginosa*, *S.abony*, *S.aureus* (with an individual count 1×10^6 cfu/ml)

10

It may therefore be concluded that 2 % solution of a solution prepared from the composition of Example 3 is capable of achieving total killing of *E. coli*, *P. aeruginosa*, *S. abony*, *S. aureus* thereby making it an effective disinfectant for eggs as well as equipment disinfection

15

Example 6: Effect of the Water quality on stability of the dissolved composition and on killing efficacy

The effect of 4 different water sources was compared for the possible effect on dissolution of the composition at three different dilutions in water at room temperature.

20

Concentration	Water quality	Test parameters					Solubility
		Hardness (ppm of CaCO ₃)	pH of solution	Hydrogen peroxide (ppm)	Peracetic acid (ppm)	Efficacy	
2 %	A	110	7.82	1785	2105	100 % Killing	dissolved
	B	305	7.93	1784	2100	100 % Killing	dissolved
	C	600	7.85	1784	2113	100 % Killing	dissolved
	D	920	7.88	1783	2089	100 % Killing	dissolved
3 %	A	110	7.89	2500	3367	100 % Killing	dissolved
	B	305	7.95	2495	3360	100 % Killing	dissolved
	C	600	7.87	2505	3345	100 % Killing	dissolved
	D	920	7.88	2506	3354	100 % Killing	dissolved
4 %	A	110	7.86	3034	3903	100 % Killing	dissolved

	B	305	7.97	3025	3889	100 % Killing	dissolved
	C	600	7.83	3033	3901	100 % Killing	dissolved
	D	920	7.87	3099	3900	100 % Killing	dissolved

5

Table 3: Effect of water quality on dissolution parameters of the composition according to Example 1. Concentration means the concentration of the dilution of the composition according to Example 1 in water. Hydrogen peroxide and peracetic acid were measured as described in Example 7. Efficacy has been measured against *P. aeruginosa* NCIM 200 cell count 10^6 cfu/ml with a contact time of 3 seconds. Solubility was assessed at 25°C and with a rotational speed of 187 rpm. Dissolved means the solution was completely dissolved after 5 minutes observation.

As shown above, water quality has no significant effect on the speed of solubilization of the composition according to Example 1. Furthermore, we have also shown that the water quality has no effect on the antimicrobial efficacy following 3 seconds of exposure.

Example 7: Stability studies of the solid composition according to the invention

Materials and Methods:

The following equipment has been used: Stability chambers having Temperature and Humidity control; Magnetic stirrer (REMI 2 ML); and pH Meter (WAVETEK 5000).

The following chemicals have been used : Standard 0.1M Potassium permanganate(A.R.) solution; Standard 0.1 M Sodium thioslphate(A.R.) solution; Dilute sulphuric acid; Potassium Iodide solution(A.R.); 10 % Distilled water.

Experimental conditions:

Temperature	30°C
Humidity	75 %
Testing intervals	Initial ,1,2,3,4,5,6 month
Temperature	40°C
Humidity	75 %

Testing intervals	Initial ,1,2,3,4,5,6 month
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5

Preparation of 2% solution from the composition of Example 1: 2 g of the composition of Example 1 were dissolved in 100 ml distilled water. Sample was stirred continuously until complete dissolution to obtain clear solution. (Solution 1)

10 Estimation of Peracetic acid and Hydrogen peroxide:

50 ml dilute sulphuric acid were transferred to the 250 ml Iodine flask. 25 ml of above prepared solution 1 were transferred to the flask followed by stirring. Titrate above solution using 0.1 M potassium permanganate solution until slight pink color appears to the solution. Note burette reading (A).

15 Add immediately 10 ml 10% potassium iodide solution to the flask.

Liberated iodine is then titrated using 0.1 M sodium thiosulphate solution until solution become colorless. Note burette reading (B).

Calculation :

Hydrogen Peroxide (ppm) = Burette reading(A) x 0.0085 x Actual molarity of KMnO₄ solution
x 1000 x 1000 divided by the volume of sample (ml) x standard molarity of KMnO₄ solution

(Each ml of 0.1 M KMnO₄ solution equivalent to 0.0085 gm hydrogen peroxide)

Peracetic acid (ppm) = Burette reading (B) x 0.0038 x Actual molarity of Sodium thiosulphate
solution x 1000 x 1000 divided by the volume of sample (ml) x standard molarity of Sodium
thiosulphate solution.

(Each ml of 0.1 M sodium thiosulphate solution equivalent to 0.0038 gm peracetic acid)

30 Table 4 shows the stability data of the composition according to Example 1 as assessed by preparing a 2% dilution in water, and assessing the pH of the solution, its peracetic acid content, as well as the hydrogen peroxide content following storage of the powder for 0 to 6 month at temperatures of 30°C or 40°C.

Time (month)	Temperature	Description	pH	Peracetic acid content (ppm)	Hydrogen peroxide content
			Data form a 2% solution		
0	30°C	White powder	8.10	2200	1785
	40°C	White powder	8.10	2200	1785
1	30°C	White powder	7.80	2113	1933
	40°C	White powder	7.80	2228	1708
2	30°C	White powder	7.80	2100	1889
	40°C	White powder	7.80	2125	1700
3	30°C	White powder	7.78	2120	1782
	40°C	White powder	7.82	2028	1754
4	30°C	White powder	7.98	2105	1701
	40°C	White powder	7.80	2066	1725
5	30°C	White powder	7.82	2107	1738
	40°C	White powder	7.80	2089	1733

5

Table 4: shows the stability data of the composition according to Example 1.

Furthermore, the stability of a composition according to Example 1 after dilution in water at 2, 3, or 4 % has been followed for 6 hours according to the method described above. The results (Table 5) show the solution is stable for at least 6 hours.

10

Concentration of Composition of Example 1 in solution at:	Time	Test parameters			
		Appearance	pH of solution	Peracetic acid (ppm)	Hydrogen Peroxide (ppm)
2 %	0 hour	Clear	7.82	2105	1785
	4 hours	Clear	7.93	2065	1785
	6 hours	Clear	8.11	2013	1755
3 %	0 hour	Clear	7.70	3367	2500

	4 hours	Clear	7.80	3167	2495
	6 hours	Clear	7.96	3054	2455
4 %	0 hour	Clear	7.70	3903	3034
	4 hours	Clear	7.92	3888	2956
	6 hours	Clear	8.26	3445	2847

5

Table 5: Stability of the composition according to Example 1 in solution.

Composition according to Example 1 in solution at:	Stage	0 hour		4 hours		6 hours	
		3"	5"	3"	5"	3"	5"
2 %	<i>E.coli</i> NCIM 2065	100 %	100 %	100 %	100 %	100 %	100 %
	<i>P.aeruginosa</i> NCIM 2200	100 %	100 %	100 %	100 %	100 %	100 %
	<i>S.abony</i> NCIM 2257	100 %	100 %	100 %	100 %	100 %	100 %
	<i>S.aureus</i> NCIM 2079	100 %	100 %	100 %	100 %	100 %	100 %
3 %	<i>E.coli</i> NCIM 2065	100 %	100 %	100 %	100 %	100 %	100 %
	<i>P.aeruginosa</i> NCIM 2200	100 %	100 %	100 %	100 %	100 %	100 %
	<i>S.abony</i> NCIM 2257	100 %	100 %	100 %	100 %	100 %	100 %
	<i>S.aureus</i> NCIM 2079	100 %	100 %	100 %	100 %	100 %	100 %
4 %	<i>E.coli</i> NCIM 2065	100 %	100 %	100 %	100 %	100 %	100 %

	<i>P.aeruginosa</i> NCIM 2200	100 %	100 %	100 %	100 %	100 %	100 %
	<i>S.abony</i> NCIM 2257	100 %	100 %	100 %	100 %	100 %	100 %
	<i>S.aureus</i> NCIM 2079	100 %	100 %	100 %	100 %	100 %	100 %

5

Table 6 : Stability of the killing activity. 100% means 100 % killing efficacy. 3" and 5" means 3 seconds and 5 seconds contact with the composition according to Example 1 at 2, 3 and 4% concentration, respectively.

10 Example 8: Antimicrobial efficacy

A 2% solution from the composition of Example 1 has been prepared as follows: 2 g of the composition of Example 1 were dissolved in 100 ml distilled water. Sample was stirred continuously until complete dissolution to obtain clear solution (Solution A).

15 To 5 ml aliquot of Solution A, 0.1 ml of bacterial culture having a density of approximately 10^6 cfu/ml was added and kept at a temperature between 18 and 20 °C with intermittent shaking. After different time intervals (Killing / Inactivation contact time of 5, 10 or 60 seconds), a loopfull was added in a test tube containing sterile nutrient broth for bacterial cultures, and sterile Sabouraud broth for fungal spp. Then all the nutrient broth tubes were
20 incubated at 37°C for 48 hours, while Sabouraud broth tubes were incubated at room temperature in the dark for 72 hours to observe growth or inactivation.

The results obtained are summarized in Table 7.

Test microorganism	Viability/Inhibition after a contact time of:		
	5 Sec.	10 Sec.	60 Sec.
<i>E. coli</i> (ATCC 25922)	N	N	N
<i>S. typhimurium</i> (NCTC 786)	N	N	N
<i>P. aeruginosa</i> (Fisher's immunotype IV)	N	N	N

<i>S. aureus</i> (ATCC 25923- MRSA)	N	N	N
<i>C. albicans</i> (ATCC 10231)	N	N	N
<i>A. Niger</i> (ATCC-16404)	V	N	N

Table 7: N means no viable organisms have been observed; V means organisms were observed. MRSA means the strain is a multi-resistant *S. aureus*.

This data confirms the speed of kill of the composition according to the present invention

Example 9: Stable Disinfectant Composition

sodium percarbonate	45 wt %
tetra acetyl ethylene diamine	22.5 wt %
anhydrous citric acid	15 wt %
sodium benzoate	0.016 wt %
anhydrous sodium acetate	9.5 wt %
sodium sulfate	6.55 wt %

The stability of a composition according to Example 9 after dilution in water at 2, or 4 % has been followed for 6 hours according to the method described above. The results (Table 8) show the solution is stable for at least 6 hours.

Table 8

Concentration of Composition of Example 9 in solution at:	Time	Test parameters			
		Appearance	pH of solution	Peracetic acid (ppm)	Hydrogen Peroxide (ppm)
2 %	0 hour	Clear	7.32	2209	1798
	4 hours	Clear	7.39	2189	1785
	6 hours	Clear	7.40	2103	1785

4 %	0 hour	Clear	7.32	3922	3036
	4 hours	Clear	7.39	3898	2965
	6 hours	Clear	7.40	3500	2879

5 CLAIMS

1. A stable disinfectant composition comprising:

- a. 40 to 50 wt % sodium percarbonate
- b. 20 to 25 wt % tetra acetyl ethylene diamine
- c. 10 to 20 wt % anhydrous citric acid
- d. 0 to 2 wt % alpha olefin sodium sulfonate
- e. 5 to 15 wt % anhydrous sodium acetate
- f. 0.01 to 0.02 wt % of an anti-corrosive agent
- g. 5 to 20 wt % of a filler.

2. The stable disinfectant composition according to claim 1, wherein the composition comprises:

- a. 42 to 48 wt % sodium percarbonate
- b. 22 to 23 wt % tetra acetyl ethylene diamine
- c. 12 to 18 wt % anhydrous citric acid
- d. 0 to 2 wt % alpha olefin sodium sulfonate
- e. 8 to 11 wt % anhydrous sodium acetate
- f. 0.01 to 0.02 wt % of an anti-corrosive agent
- g. 5 to 11 wt % of a filler.

3. The stable disinfectant composition according to claims 1 or 2, wherein the filler is sodium sulfate.

4. The stable disinfectant composition according to any of the claims 1 to 3, wherein the anti-corrosive agent is sodium benzoate.

5. The stable disinfectant composition according to any of the claims 1 to 4, wherein the composition comprises:

- a. 44 to 46 wt % sodium percarbonate
- b. 22 to 23 wt % tetra acetyl ethylene diamine

- 5 c. 14 to 16 wt % anhydrous citric acid
 d. 0 wt % alpha olefin sodium sulfonate
 e. 9 to 10 wt % anhydrous sodium acetate
 f. 0.01 to 0.02 wt % of sodium benzoate
 g. 5 to 8 wt % of sodium sulfate.
- 10
6. The stable disinfectant composition according to any of the claims 1 to 5, wherein the composition is in a tablet form.
7. The stable disinfectant composition according to any of the claims 1 to 5, wherein the composition is packaged in a sachet or a pouch.
- 15
8. The stable disinfectant composition according to claim 7, wherein the sachet or the pouch is made of water soluble material.
- 20
9. The stable disinfectant composition according to any of the claims 1 to 8, wherein the composition is dissolved in water at a dilution of 1 to 5 %.
10. The use of a composition according to claim 9, as a disinfectant for spraying eggs prior embryo vaccination, for spraying egg vaccination equipment, and/or for flushing of vaccination equipment.
- 25
11. The use according to any of the claims 1 to 8, wherein the composition is dissolved in tap water at room temperature and at a dilution of 2 to 3 %.
- 30
12. The use according to claims 9 to 11, wherein the disinfectant is effective in killing *E. coli*, *P. aeruginosa*, *Staphylococcus* species and/or *Salmonella* species.
13. A method of disinfecting eggs and hatchery equipment, including vaccination equipment, comprising the steps of dissolving a composition according to any of the claims 1 to 8, in tap water at room temperature and at a concentration of 1 to
- 35

- 5 5%, and contacting the disinfectant solution for 3 to 5 seconds with surfaces to be treated.