

# REDUCING THE NUMBER OF BACTERIAL COLONIES USING ECOCID® S (POTASSIUM PEROXYSULPHATE BASED DISINFECTANT) AT SMALL ANIMAL CLINIC

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**Abstract:** A clinical study has been conducted to test the efficacy of Ecocid® S, a biocidal agent. The active substance is potassium peroxy sulphate and is used in clinical practice after the mechanical cleaning of various surfaces that act as potential sources of infection transmission. We determined 29 swabbing points, from which 87 samples were collected with cotton swabs. Swabs were submitted for microbiological testing to evaluate microbial contamination before cleaning, and before and after disinfection with Ecocid® S. We submitted 63 swabs from 21 swabbing points for further statistical analysis. Five swabs were excluded because the presence of bacteria in the swabs before disinfection had not been determined. The clinical study on the efficacy of Ecocid® S disinfectant showed that it is effective with an average reduction in contamination of 95.75%. The disinfectant was also active with a significantly reduced time of action: it was removed with dry paper towels from all sampling points, except the floor scales, only 5 to 10 minutes after application. The time required for the proper preparation of examination tables and other equipment in clinical practice is of vital importance for a smooth workflow.

**Key words:** animals; disinfection; potassium peroxy sulphate; Ecocid® S

## Introduction

The standard cleaning protocol is applied at Small Animal Clinic to maintain suitable hygiene conditions following the professional recommendations and good clinical practice (1). All surfaces should be made of materials suitable for cleaning with disinfectants and for mechanical wet cleaning to prevent transmission of pathogens from one patient to another, and in case of zoonoses, from animal patients to humans.

A clinical study has been conducted at the Small Animal Clinic to test the biocidal agent Ecocid® S in clinical conditions. Ecocid® S belongs to a group of oxidising disinfectants. Different studies have confirmed the efficacy and safety of Ecocid® S under laboratory conditions<sup>4,5</sup>(2).

The active substance of Ecocid® S is potassium peroxy sulphate. Its efficacy is increased by added surfactants, organic acids and an inorganic buffer system. It has been proven effective against many infectious microorganisms such as viruses, bacteria and fungi (3, 4, 5, 6, 7). Because of its special composition, Ecocid® S guarantees good contact with the cell surface and acts on most cell elements, the cytoplasmic membrane, the cytoplasm and the nucleus. By acting on the nucleus, it causes the destruction of the pathogen's genetic material and therefore prevents horizontal and vertical disease transmission (8).

<sup>4</sup>Zorman Rojs O. Ugotavljanje virucidnega delovanja Ecocid® S na virus aviarne influence H5N1, laboratorij za kužne bolezni perutnine, Univerza v Ljubljani, Veterinarska fakulteta, Ljubljana, 2006

<sup>5</sup>Zorman Rojs O. Comparative testing of the efficacy of the disinfectant Ecocid® S against gumboro disease virus isolates of different pathogenicity, Institute for poultry health and protection, University of Ljubljana, Veterinary faculty, Ljubljana, 2007.

The study aimed to confirm the efficacy and safety of Ecocid® S in clinical conditions at small animal clinic using the standard protocol of cleaning and disinfection of the most exposed areas of the clinic.

## Material and methods

### *Cleaning and disinfection procedure*

The mechanical cleaning procedure for all selected surfaces was carried out according to the operating procedure of the clinic, based on the good clinical practice and according to FECAVA Key Recommendations for Hygiene and Infection Control in Veterinary Practice (1). Potentially critical sites as regards microbial contamination and possible transmission routes of infections were determined and included in the study. After mechanical cleaning with a neutral detergent solution with water and paper towels, the surface of each item was disinfected with freshly prepared 1% Ecocid® S solution, according to the recommendations of the manufacturer (9). Approximately 100 ml of the solution per m<sup>2</sup> was used and left to act for approximately 5–10 minutes. Only the floor scale had a 30-minute contact time.

### *Sampling*

A sterile cotton bud was dipped into a tube of 5 ml 0.1% peptone salt solution (Proteose Peptone 1.0 g/L (Biolife Italiana Srl, Milan, Italy), NaCl 8.5 g/L (Merck KGaA, Darmstadt, Germany)). The surface of each 20 cm<sup>2</sup> marked spot was swabbed in two directions. A sample was taken immediately before and after sanitation (cleaning, disinfection) from each sampling site. After disinfection, each sampling site was dried with paper towels before swabbing to neutralise any biocidal residues on the surface and to prevent any further biocidal action on microorganisms in the samples before the laboratory analysis. After sampling, the swabs were refrigerated and brought to the laboratory within two hours of collection.

Sampling sites were determined according to the highest exposure rate of the equipment (Table 1).

### *Microbiological method*

The method for the enumeration of microor-

ganisms was used to evaluate the surface contamination levels before cleaning and before and after disinfection. Swab samples were homogenised, diluted where needed and inoculated into Petri dishes. Non-selective solid medium (Tryptic glucose yeast agar, Biolife) was poured, allowed to solidify and then incubated for 72 hours at 30 °C. A sample with a known concentration of *Bacillus subtilis* subsp. *spizizenii* WDCM 00003 was tested in parallel to other samples as a quality control. Results were calculated based on counted colonies and expressed as the number of colony forming units per 20 cm<sup>2</sup> (CFU/area).

### *Data processing and report preparation*

Basic statistical methods were applied in data processing (calculation of percentages-reduction of number of microorganisms), and the following tests were applied in data analysis: the  $\chi^2$  test (chi-squared test) for the comparison of the number of swabs based on the given criteria (80% reduction and 95% reduction of microorganisms). The number of microorganisms after cleaning and after disinfection were compared. Before the data were processed, values for total colony forming units were converted to logarithmic values. Analysis of variance (ANOVA) and the t-test were used to establish the difference between group means. Tukey's test was applied if differences between group means were statistically significant.

## Results

We presented Ecocid®S action test results by individual swabbing sites (Table 1). The effectiveness of disinfection was evaluated according to the difference between the evaluated contamination (CFU/20 cm<sup>2</sup>) before cleaning and before and after disinfection expressed in logarithmic values ( $\log_{10}$ ) and percent. When reduction was  $\geq 1.0 \log_{10}$  CFU/20 cm<sup>2</sup>, contamination decreased by 90.0% or more. If reduction was  $\geq 2.0 \log_{10}$ , the drop was at least 99.0%, while the 99.99% or more decrease was recorded for reductions  $\geq 3.0 \log_{10}$ .

The staff collected 87 swab samples from 29 swabbing points. Of these, 63 swabs from 21 swabbing points were submitted to the ensuing statistical analysis. Five swabs were not included in the statistical analysis because the presence of bacteria

**Table 1:** Source data – number of microorganisms (CFU/20 cm<sup>2</sup>) for swabbing points and testing phases

Disinfection area	Sample	1	2	3
Cage top panel, presurgical room	1	0	0	0
Cage top grate, presurgical room	2	65	1200	10
Cage bottom panel, presurgical room	3	0	4600	0
Cage bottom floor, presurgical room	4	600	3000	25*
Cage bottom floor (cat), hospital	16	220	2300	0
Cage bottom panel (cat), hospital	17	15*	6000	0
Cage bottom floor (dog), hospital	18	150000	12000	10*
Cage bottom panel (dog), hospital	19	2300	85000	10*
Examination table, cardiology ultrasound	5	25*	40*	0
Table, surgery room no. 3	6	65	2200	0
Table, presurgical room	7	15*	15000	0
Table, dentistry	8	0	15000	0
Examination table, exam room no. 106	20	15*	6000	0
Examination table, hospital	21	180	85	0
Examination table, ultrasound	24	460	0	0
Examination table, X-ray room	26	55	10*	10*
Examination table, dermatology room	29	1400	15000	20*
Transport table	25	450	30*	0
Thermophore (cat)	12	15*	0	0
Thermophore (dog)	28	140	25*	20*
Scale, reception	14	950	2300	60
Scales, exam room	15	550	8000	0
Inhalation chamber, panel	22	1100	15	0
Inhalation chamber, connectors	23	0	0	0
Laminar airflow bench for preparing cytostatic agents	27	40	15000	15
Tracheal tube (cat), orthopaedic	9	750	0	0
Tracheal tube (dog), dentistry	10	85	10	0
Tracheal tube (dog), orthopaedic	11	0	0	0
Tracheal tube (cat)	13	20*	0	0

**Legend:**

1 – Number of microorganisms before cleaning (CFU/surface)

2 – Number of microorganisms before disinfection (CFU/surface)

3 – Number of microorganisms after disinfection (CFU/surface)

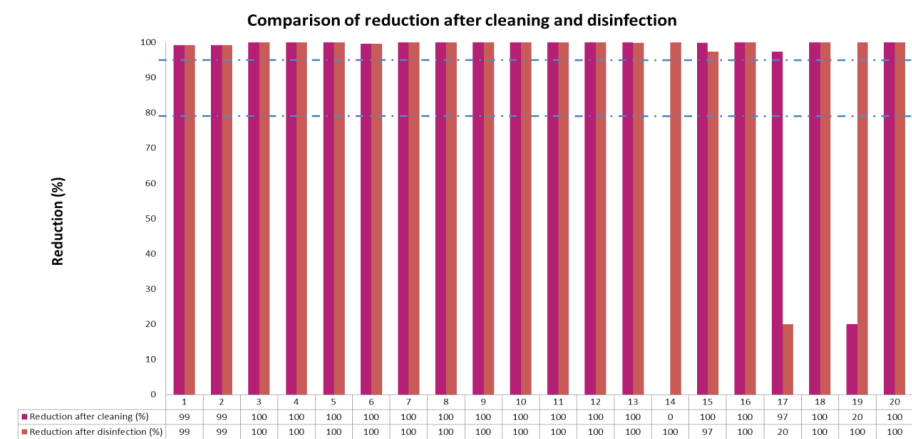
\* - Estimated number – low counts (&lt; 10 CFU/plate) - precision of the result is low and the result is reported as estimated

Note regarding the 0 value: Number of microorganisms &lt; 5 CFU/surface (values under the detection limit) were regarded as 0.00

in the swabs before disinfection had not been determined (< 5 CFU/surface). The number of microorganisms was converted to logarithmic values. For statistical processing, results reported as < 5 CFU/surface, i.e. below the detection limit, were assigned a value of 0.00 log<sub>10</sub>, corresponding to 1 CFU.

Swabs collected from cages showed that the average decrease in the contamination level after disinfection was statistically significant at 99.64% (P < 0.01). The differences in contamination lev-

el before cleaning and after disinfection were also statistically significant (P = 0.006). Swabs collected from tables showed that the number of microorganisms after disinfection decreased on average by 88.87%. Reduction in contamination after disinfection compared to before disinfection was statistically significant (P < 0.001). Reduction in contamination after disinfection in comparison to the number of microorganisms before cleaning was also statistically significant (P = 0.004).



**Figure 1:** Reduction in contamination (%) after cleaning and after disinfection on all tested surfaces

The number of microorganisms after disinfection of tracheal tubes decreased on average by 100%. Reduction in contamination after disinfection in comparison to the level before cleaning ( $P = 0.011$ ) indicated that the complete sanitation procedure was effective.

Results of the swabs collected from scales showed a reduction in contamination levels after disinfection, but due to the small number of samples, it could not be proven statistically. Swabs collected from only two thermophores showed that cleaning reduced the number of microorganisms: in one case contamination levels after cleaning diminished completely and in another significantly. Comparison of contamination levels before cleaning and after disinfection indicated that cleaning had a significant impact on the entire sanitation procedure. Swabs collected from other equipment (an inhalation chamber panel and a biological safety cabinet for preparation of cytostatic agents) showed that the number of microorganisms after using Ecocid® S decreased on average by 100% (an inhalation chamber), and by 99.90% (a laminar flow).

According to the statistical analysis of 21 swabs out of total 24, it appears that the decrease in contamination level after disinfection was statistically significant ( $P < 0.05$ ). Three samples were excluded because all microorganisms had already been removed by cleaning. Contamination after disinfection was reduced by 91.2% (SE = 5.9%) on average compared to contamination after cleaning. In one instance (a thermophore used in dogs) contamination after cleaning was reduced by 0% in comparison to disinfection. In this case, the number of microorganisms was very low (25 after cleaning, and 20 after disinfection), even though

this may be attributed to the uneven surface of the thermophore, which features a ribbed rubber design. In 20 remaining swabs, contamination was reduced by almost 96% (95.75%).

There were no statistically significant ( $P = 1.00$ ) differences between the compared criteria of reduction effectiveness (95% or 80%) regarding the number of microorganisms (Figure 1).

## Discussion

The investigation showed that Ecocid® S is effective in clinical conditions if cleaning and disinfecting protocols applicable at the clinic are followed and if the preparation is made in accordance with professional guidelines and good clinical practice (1). Microbial contamination decreased on average by 91.2% after disinfection with Ecocid® S, which is comparable with previous findings<sup>6,7,8,9</sup>. This represents a statistically significant reduction at  $P < 0.05$ , which was also confirmed by separate analyses for individual sampling points on cages, examination tables, and tracheal tubes. Cages and tables are made of stainless steel and the removable sub-floor grate is plastic-coated, so all surfaces are smooth and good hygiene is already maintained with mechanical cleaning. In certain cases, the number of microorganisms went up, which

<sup>6</sup>Ščuka L. Statistical analysis of the efficacy and safety study of the Ecocid® S in comparison with reference product, Ljubljana, 2008. Report: 1–10.

<sup>7</sup>Juršič R, Ščuka L. Ugotavljanje učinkovitosti dezinficijskega sredstva Ecocid® S v praktičnih pogojih na perutninskih farmi. Report: 1–7.

<sup>8</sup>Poročilo o testu delovanja biocidnega sredstva Ecocid® S v prašičerejskem objektu, Univerza v Ljubljani, Veterinarska fakulteta, February 2007.

<sup>9</sup>Gruntar I. Poročilo o baktericidni aktivnosti (prEN 1656) produkta Ecocid® S, Inštitut za mikrobiologijo in parazitologijo, Univerza v Ljubljani, Veterinarska fakulteta, Ljubljana 2007.

could be attributed to using the cleaning agent in a sprayer. It is possible that the spray, which includes microorganisms, forms an aerosol that settles on the surface again. Contamination can therefore only be reduced by applying a disinfectant. Even though we removed the disinfectant with dry paper towels within 5 to 10 minutes after spraying, it obviously acted long enough to achieve the required result. The suggested exposure of the disinfectant used in the study is 30 minutes (9). We believe that the reduced time of action is exceptionally important for clinical practice, because it allows for much faster patient flow and a smooth workflow.

Ecocid®S is an effective biocide and can also be used for tracheal tubes of smooth non-porous plastics after cleaning by rinsing with drinking quality water. We believe that any residual disinfectants used for disinfection of tracheal tubes coming in direct contact with the mucous surface of the respiratory tract should be removed thoroughly with drinking quality water.

Differences in results of swabs collected from scales were considerable and can be considered significant, although the swabs were only collected from two scales. Cleaning and disinfection were slightly less effective with the floor scale, which was expected because of its non-slip PVC lining with a rough textured surface compared to table scales, whose surface is made of smooth plastic material. Cleaning of floor scales generally takes place twice a day and more often if patients discharge (defecate, urinate, or vomit) on the device. More microorganisms from soiling are expected to accumulate on the lining, which cakes after a while. Only when this had happened, did we leave the disinfectant to act for 30 minutes. However, the extended time period was not sufficient to eliminate the presence of microorganisms. We believe that the extended time of action helped dissolve caked soiling, because the preliminary mechanical cleaning protocol was the same as with smooth surfaces. To reduce contamination more efficiently, it would be necessary to optimise mechanical cleaning. We nevertheless believe that the disinfection protocol for the floor scales is satisfactory because patients generally only stand on the lining. This does not represent a major hazard if the number of microorganisms is as low as established during our investigation. We obtained similar results using the same cleaning and disinfection protocol on thermophores,

which are often used with patients during and after general anaesthesia. Direct contact with patients is generally avoided when using thermophores. Because their surface is uneven, they must be appropriately cleaned whenever they come into contact with a patient, with drinking quality water and disinfectant. To remove any residual disinfectant that might potentially cause irritation, it is recommended that they are rinsed with drinking quality water after disinfection.

Analysis results of other sampling points (the inhalation chamber and the biological safety cabinet for preparation of cytostatic agents), selected as a potential source of transmission of pathogens also show that cleaning and disinfection procedures were appropriate.

Efficacy of Ecocid® S biocidal preparation in practical clinical conditions at the Small Animal Clinic in all places at the structure and on all selected surfaces fully met the required performance threshold of decreasing contamination on average by 1 log<sub>10</sub> CFU/20 cm<sup>2</sup> (i.e. the contamination level was reduced at least by 90%). There are limited data on hospital-associated infections and only a few studies on optimal cleaning and disinfection procedures in small animal clinical practice (10, 11).

An important limitation of the study is that we did not have the opportunity to test the efficacy on selected and important pathogens, although the product was tested on some of these in laboratory conditions.

## Conclusion

The study showed that Ecocid® S disinfectant is effective in practical clinical conditions with an average 95.75% reduction in microorganisms compared to samples before and at the end of the disinfection process. The disinfectant was also effective when the time of action was significantly shorter, as it was wiped clean with dry paper towels from all sampling points except the floor scales only 5 to 10 minutes after application. The time needed for the entire process of proper disinfection between individual patients is of utmost importance for a smooth clinical workflow. We believe that Ecocid® S can be successfully used even for the same equipment, such as tracheal tubes. When it is rinsed off with drinking quality water, it does not cause any irritation in animals.

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## ZMANJŠANJE ŠTEVILA BAKTERIJ PO UPORABI RAZKUŽILA ECOCID® S (RAZKUŽILO NA OSNOVI KALIJEVEGA PEROKSISULFATA) V PROSTORIH KLINIKE ZA MALE ŽIVALI

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**Povzetek:** S klinično študijo smo želeli ugotoviti učinkovitosti biocidnega razkužila Ecocid® S. Aktivna snov v razkužilu je kalijev peroksisulfat, ki se na klinikah uporablja za razkuževanje različnih površin, ki predstavljajo ključna mesta za prenos okužb, po njihovem mehaničnem čiščenju. Določili smo 29 vzorčnih mest, na katerih smo z uporabo bombažnih brisov odvzeli 87 vzorcev. Z mikrobiološkim testiranjem smo ugotavljali stopnjo kontaminacije pred čiščenjem, ter pred in po razkuževanju z Ecocid® S. Za statistično obdelavo smo uporabili 63 rezultatov z 21 vzorčnih mest. Pet rezultatov je bilo izločenih iz obdelave, ker je bila že pred razkuževanjem stopnja kontaminacije pod mejo detekcije uporabljene metode. S klinično študijo učinkovitosti razkužila Ecocid® S smo ugotovili povprečno 95,75 % zmanjšanje števila kontaminantov po uporabi razkužila. Razkužilo je bilo učinkovito tudi ob skrajšanem času delovanja le 5 do 10 minut po nanosu. Zaradi zagotavljanja tekočega dela na kliniki je izredno pomemben čas, ki je potreben za ustrezno pripravo površin in druge opreme za pregled živali, zato je razkužilo Ecocid® S primerno za uporabo na veterinarskih klinikah, saj hitro in učinkovito zmanjša bakterijsko kontaminacijo.

**Ključne besede:** živali; dezinfekcija; kalijev peroksisulfat; Ecocid® S