# **Sequani's environmental footprint**

## REECE READING

Sequani

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### Background

All commercial organisations must comply with prescribed water quality/discharge limits to operate under their trade effluent licences granted by their water suppliers (Table 1).

Parameters	Unit	Range
pH Level	1 (acid) – 14 (alkali)	6.0 - 10.0
Phosphorus Level	mg/L	0 – 25

 Table 1. Trade Effluent Ranges, Severn Trent Water.

Limits ensure the quality of our natural waterways and ensure the biodiversity they contain and prevent release of potentially harmful substances.

As a result of an altered sample point during the building works for our new biomedical facility we observed that while complying with the requirements for site release there was an opportunity to improve on 2 measures of water quality: phosphate levels and pH from the discharge directly from a rodent unit cagewash area (image 1).



Image 1. New biomedical facility building.

### Aim

Ensure the effluent that is directly discharged from our cagewash machines is phosphate free and pH neutral.

#### Method

Current commercially supplied detergent is phosphoric acid based (phosphate levels of >30%, pH 1.8 to 2.2); historically, used because of its excellent degreasing and de-mineralisation properties especially useful for urine and hard water staining.

In collaboration with the cagewash manufacturer, we sourced a phosphate-free, citric acid-based detergent that would not cause any technical issues with our cage wash equipment and assessed:

- Visual cleanliness of the soiled rodent cages.
- Microbial monitoring conducted by; aseptically swabbing an item on completion of cycle and placing the swab in nutrient broth, incubating the broth under shaking at 100 rpm, 37°C for 16 to 24 hours, treating an airdried drop of the broth with a gram stain kit, counting the gram positive and gram-negative stained bacteria under a micro-scope.
- Method of neuralisation of the cagewash effluent was developed in collaboration with the cagewash manufacturer the cagewash machines have different discharge systems (image 2).



Image 2. Sump system.

- Discharge of effluent into a sump before full discharge to drain.
- Direct discharge to drain.
- To assess effectiveness of the neutralisation methods, we sampled outflow from the cagewashes at point of entry into the main drain i.e. only cagewash outflow being sampled.
- pH was assessed in the samples on the same day of sampling using a standard laboratory pH meter, calibrated prior to measurement.

### **Results**

 Switch to a citric acid-based detergent ensured both visually clean and microbiologically sanitised cages (see Table 2) and removed all phosphate.

Parameters	Results (Pass/Fail)
Gram Negative Rods	Pass
Gram Positive Cocci	Pass
Cagewash Acceptability Limits	Pass
Adequately Sanitised	Pass
Working Satisfactorily	Pass

Table 2. Cage sanitisation.

 Retrofitting of a sump-based neutralisation system, Image 2 and 4, and an in-line neutralising system, Images 3, 5 and 6 achieved pH neutral water discharges, see Figure 1.



Dosing pumps (1). Sump located underneath machine (2).

Image 4. Actual Sump System.







Image 3. In-line drainage system.



Panel for pH monitoring (Neutraliser Unit) (1). Dosing pumps (2). pH algorithm monitor (within panel) (3).

Images 5 & 6. Actual in-line system.

#### **Summary**

- Switching to phosphate-free detergent does not negatively impact the sanitising of Cage wash process.
- Retrofitting of neutralising infrastructure to cage washes with or without sumps are effective at pH neutralising effluent at point of discharge.