

varieties of oranges, and Dancy tangerines, to determine the reliability of the counteractant under various conditions and, at the same time, to evaluate the effect of the mixture on decay control. Approximately 30,000 individual fruits have been under observation. In no case has hexamine failed to prevent peel burn, even under severe conditions of treatment, namely, when the fruit was dipped in 2% DOWICIDE A for 2 min at 100°F and not rinsed after treating (Fig. 1). In one experiment a solution containing 3% DOWICIDE A and 1.5% hexamine was used as a dip, and the treatment made as just described. No trace of burn was produced on oranges so treated, whereas 1.5% DOWICIDE A without the counteractant caused severe burn on the same lot of fruit.

TABLE 1

Treatment	Total decay, %*
Controls, untreated	24.4
DOWICIDE A, 2%	4.9
DOWICIDE A, 2% + hexamine 1% .	3.7

* Stem-end rot and mold.

Data obtained from oranges held in storage for 3 weeks show that the addition of hexamine to the DOWICIDE A solution does not interfere with its fungicidal action on the organisms causing stem-end rot and mold decay. The mean values for 8 experiments are presented in Table 1. In all 8 experiments the oranges were subjected to an ethylene coloring treatment for 60-90 hr before receiving the fungicidal dip. This hastens the onset of stem-end rot decay and makes its control more difficult. As shown in Table 1, good protection against decay was also afforded by DOWICIDE A used alone, but in all cases the fruit was badly burned and of no value.

In addition to the results given in this paper, extensive data, to be published elsewhere, have been accumulated in respect to the factors involved in this DOWICIDE A-hexamine treatment. These data have shown that excellent control of both stem-end rot and mold decay are obtained when oranges are dipped in a solution containing 2.0% DOWICIDE A and 1.0% hexamine for 2 min at 100°F and not rinsed following treatment. A number of runs made in commercial packing houses have also shown a high degree of decay control without injury to the fruit peel.

An explanation of the remarkable effect of hexamine in preventing injury to plant tissues—in this case fruit peel—by DOWICIDE A, without interfering with fungicidal action, remains for future work. However that may be, the fact remains, and promises to give us a means of stopping the enormous economic loss from citrus fruit decay. We suggest also that it will find application in other instances where the use of DOWICIDE A is indicated.

References

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A Mouth-swabbing Technique for the Laboratory Mouse

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Although mouth-swabbing techniques are widely used, the literature contains no reports of the application of such methods to small laboratory animals. Inherent difficulties are directly related to the small size of the animal. Two of the most serious handicaps are possible injury to the animal and contamination of the swab by the animal's face and paws. A satisfactory technique should therefore consist of an adequate appliance and a method of handling that will insure reproducible samples without injury to the animal or contamination of the swab.

An appliance and a method that meet these requirements have been devised and are herewith described.

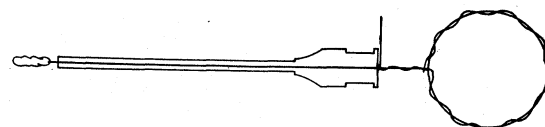


FIG. 1. Appliance diagrammed to show relations of parts when in mouth.

The device consists essentially of a cannula with a trochar and is analogous to the West nasopharyngeal swab (1). A 2-in., 18-gauge needle, which has had its end squared and dulled, is used as the cannula; the trochar is made of stainless steel wire of 0.013-in.

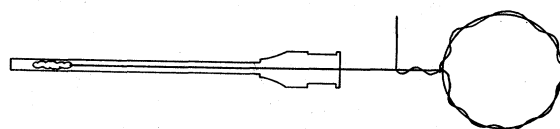


FIG. 2. Swab drawn back into needle for introduction to mouth.

diameter. The distal end of the wire, which can be projected beyond the end of the needle, is serrated and wound with a few strands of cotton to serve as the swab proper; the proximal end bears a stop and a loop (Fig. 1). The stop prevents the swab from being projected more than the predetermined $\frac{1}{4}$ in. beyond the end of the needle, and the loop allows the operator's index finger to manipulate the swab. To insure sterile dry swabs, it

¹ The opinions and assertions contained in this report are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

has been found most practical to autoclave first and then oven-dry the devices in packets of five.

Introduction of the appliance into the mouth of the animal is based on the observation that a mouse almost invariably will reach with his mouth and hold with his teeth any small article that is presented before him. In practice, the mouse is held in the conventional manner in the left hand, and the needle is held at its hub by the thumb and middle finger of the right hand with the index finger in the wire loop. With the swab drawn back into the needle (Fig. 2), the device is held before the

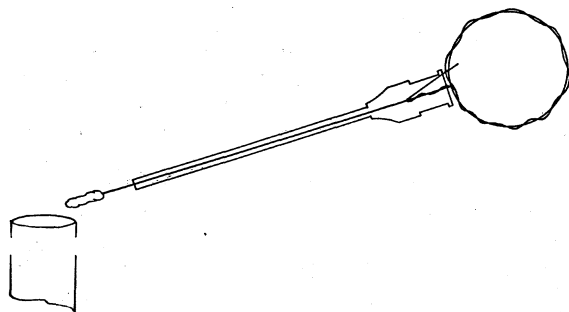


FIG. 3. Stop forced and cotton ready to be dropped into diluting fluid.

animal's face. When he reaches for it, he usually will grasp the end of the needle and begin to chew on it. At this point, the operator's index finger can push the swab gently out of the needle and into the animal's oropharynx. The stop will inform the operator when the swab is correctly positioned in the animal's mouth and will thus prevent injury caused by pushing the swab too far into the throat. It has been our experience that merely holding the swab in the mouse's mouth and allowing it to be tongued for 5 sec will insure a sample that will be as reproducible as any obtained by more elaborate manipulation. Before removal of the appliance from the animal's mouth, the swab is drawn back into the needle.

After removal, the swab is pushed about $\frac{3}{4}$ in. beyond the end of the needle by forcing the stop. Sterile forceps are used to pull the cotton off the wire and drop it into the diluting fluid (Fig. 3). After shaking the swab in the diluting fluid, an aliquot is plated on the culture medium of choice.

This appliance has been used with the Namru strain (2) of albino mice in studies to determine the relation between the oral flora, exposure to airborne pathogens, and the resulting degree of infection. In one exploratory test of the applicability of this technique, daily mouth swabs were cultured from 27 normal mice and from 20 mice that had been exposed for 15 min to a cloud containing *Streptococcus zooepidemicus* in a concentration of approximately 3×10^8 organisms per liter of air. The swabs were shaken in 10 ml of nutrient broth, and 0.1 ml aliquots were dallied on 5% cow blood agar and incubated at 37° C for 24 hr. Of the normal animals, 90% produced less than 3×10^3 β -hemolytic colonies per swab during the 3-week experimental period. On the other hand, counts from the infected animals showed an up-

ward trend until, at the end of the 3 weeks, 11 of the 12 survivors produced between 2×10^4 and 2×10^5 β -hemolytic colonies per swab. The 8 nonsurvivors died within 5 days after exposure; their counts during this period were comparable to those of the survivors sampled simultaneously. In addition to colonies showing β -hemolysis, other colonial types were noted on most plates.

The fact that a trend could be discerned in the infected animals, together with the observation of various colonial types from both normal and infected animals, indicates that this mouth-swabbing technique should allow satisfactory sampling of the oral flora of mice. Further studies will be reported at a later date.

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A Method for Artificial Insemination in Viviparous Fishes¹

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The practice of successful artificial insemination in man and in certain domesticated mammals, particularly horses and cattle, is well known, and the artificial insemination of bees is a common practice of apiculturists (1). The technique has also been used effectively in poultry for the study of intraspecies competition among sperm of different breeds (2).

Methods for the external artificial fertilization of a variety of invertebrate eggs and those of a number of oviparous poikilothermic vertebrates have been widely employed by experimental embryologists, pisciculturists, etc. However, the artificial insemination of viviparous cold-blooded vertebrates (or poikilothermic vertebrates which lay fertilized eggs) has never, to the writer's knowledge, been previously reported.⁴

In the course of studies on the sexual-isolating mechanism between the sympatric species of poeciliid fishes, *Platyphocilus maculatus* and *Xiphophorus hellerii* (3, 4, 5), interspecies sperm competition appeared to be a significant factor. Cross-species matings and the pro-

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⁴ After this paper went to press, it was called to the writer's attention that a paper in Russian (10) reports hybridization between *X. hellerii* and *P. maculatus* by means of artificial insemination.