

Teaching during the COVID-19 Pandemic: Sharing Results and Data Obtained from the Ames Test

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INTRODUCTION

The Ames test is an assay that is used worldwide to test the potential mutagenicity of different substances, from chemicals in commercial use to environmental samples and body fluids (1–3), and it is also used in the laboratory classroom setting (4, 5). We have been successfully teaching the Ames test to undergraduate biology and biochemistry students since 2011 (6, 7). It is low tech and relatively simple to put into practice, and it allows students to come into contact with real-world scenarios. Lately, we have focused on performing the Ames test with environmental samples which were relevant to our students, since they were collected from locations with which our students could easily relate, helping to create a more meaningful educational experience (7).

The COVID-19 pandemic brought many changes, including the way we, as instructors, were able to carry out our educational curricula, since access to laboratory classrooms was not always possible. While COVID-19 restrictions are still in place and thus access to laboratory classrooms is limited or null, instructors can use our online resource so

that students can perform the test remotely or under hybrid circumstances and thus can “conduct” the Ames test online, without the need to set foot in a laboratory classroom. When COVID-19 restrictions are lifted and access to laboratory classrooms is permitted, instructors can follow the procedures we describe and compare their results with ours, which appear in Results and Discussion. The flowchart in Fig. 1 describes the choices available to instructors.

PROCEDURES

As described previously (7), our students repeatedly proposed working with sediment samples taken from different locations along the bay of Montevideo and analyzing their potential mutagenicity by means of the Ames test. Since we considered this to be an excellent opportunity for our students to make a link between their everyday environment and routine laboratory assays, we developed a specific protocol for sediment samples (7), which is included in the supplemental material.

For instructors who are able to access their laboratory classrooms, we propose they carry out the Ames test and, should they be interested, use our protocol for processing sediment samples. Once they have obtained the corresponding results, they can compare them with the data sets we share in Results and Discussion.

SAFETY ISSUES

This is a “dry lab” exercise with no microbial agents or hazardous materials. However, if instructors decide

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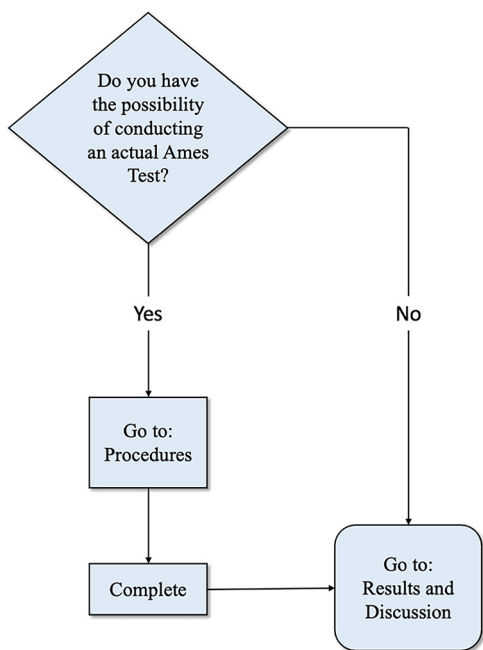


FIG 1. Flowchart to guide instructors as to how to use this resource.

to conduct the activities, the corresponding safety issues are described, together with the procedure, in the supplemental material. Said safety issues adhere to ASM Laboratory Safety Guidelines.

RESULTS AND DISCUSSION

In 2019, samples were taken from two very different locations: (i) two recreational areas and (ii) two more-industrialized areas. All sediments were processed and subsequently analyzed by the Ames test (see the supplemental material). Both of the samples from recreational areas came from shoreline beaches, one from a very popular and frequently visited location and

the other from a more secluded and less frequented site. The samples from the industrialized areas came from two different streams, one near a meat-processing plant and the other close to a tannery. Results from both areas (recreational and industrialized), together with negative and positive controls for the test, are shown in Fig. 2 to 4.

For instructors who carry out the Ames test with their own samples, the results presented below can be used to compare and contrast, and thus broaden their classroom discussions. For instructors who are not able to carry out their own Ames test, we propose the following: students can be divided online into five groups, i.e., two for the recreational areas, two for the industrialized areas, and one for the controls. Each group is sent the corresponding set of photographs. Recall that the Ames test relies on mutants of *Salmonella typhimurium* that are deficient for the synthesis of the amino acid histidine (His^-) and subsequent quantification of the His^+ revertants induced by exposure to the substance under study (8, 9). Before counting the colony forming units (CFU), a discussion with the whole class regarding expected outcomes is highly recommended. By definition, for a substance to be considered mutagenic, it must at least double the CFU count of the negative control.

Once the students have been assigned online to a group, the counting process can begin. As the results from all five groups are received by the instructor, they can be uploaded to a table (Table 1). When the table is completed, an online discussion should be conducted.

Suggestions for faculty are as follows: (i) Discussing the importance of adequate controls for the assay, and their significance, is essential. (ii) The results from recreational area 2 highlight the importance of performing environmental analyses in triplicate, since the count for one of the plates is somewhat different from the others. This could be discussed online, and other examples could be shared. (iii) The results obtained from industrialized area

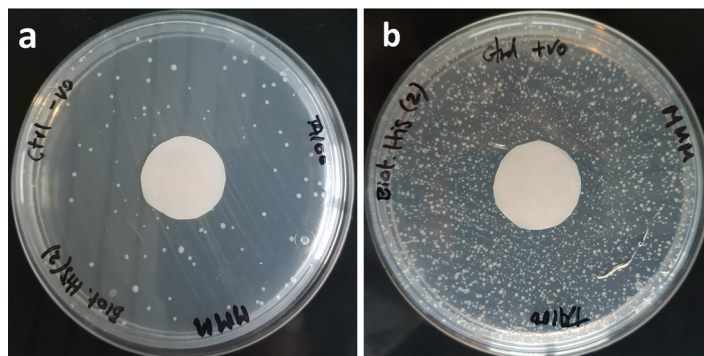


FIG 2. Controls for the Ames test. (a) Negative control. (b) Positive control.

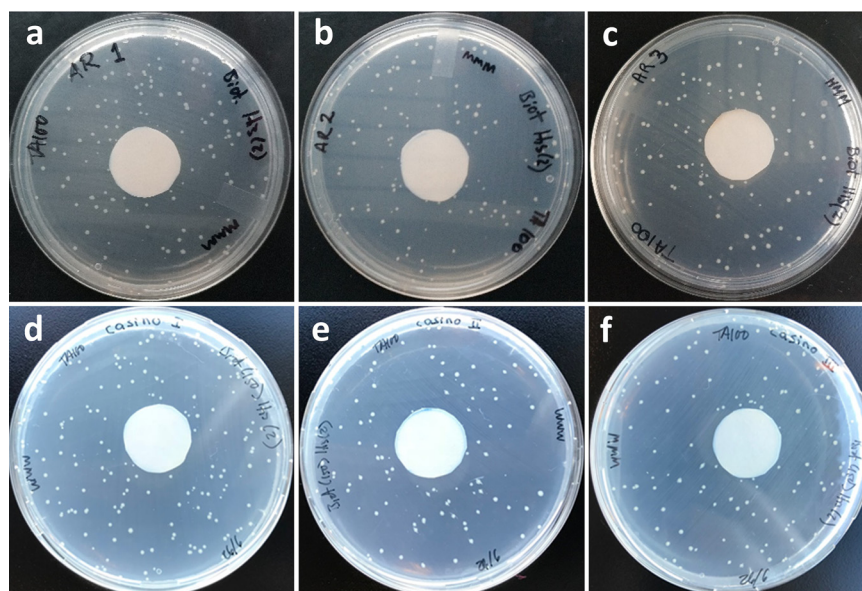


FIG 3. Results for recreational areas (triplicate samples). (a, b, and c) Samples from location 1. (d, e, and f) Samples from location 2.

2 are very interesting. There are clearly substances (one or more) interfering with the growth of the *Salmonella* indicator strain. The differences between growth interference and growth inhibition could be analyzed and discussed online. (iv) Online discussions of possible follow-up procedures for the two cases mentioned above (numbers ii and iii) make for excellent starting points for problem-based learning.

Additionally, problem-solving skills can be worked on by means of analysis and interpretation of Ames test data sets. These exercises can either be handed out to students

as a take-home assignment or can be discussed online. We have included three such exercises, together with the answer key, in the supplemental material. The following is an example.

Exercise 1: Water from a river close to an industrial area is being analyzed using the Ames test. The test was done in triplicate, with the corresponding controls. After the standard 48-h incubation period, the results obtained are as follows (Table 2). What conclusions can be drawn from the data? How do you substantiate your answer?

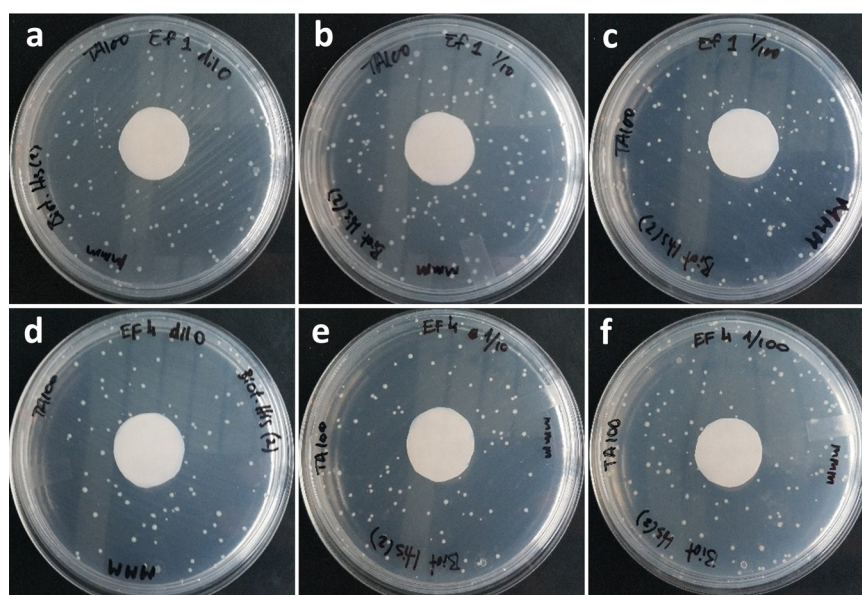


FIG 4. Results for industrialized areas. (a) Undiluted sample for location 1. (b) Sample diluted 1:10 for location 1. (c) Sample diluted 1:100 for location 1. (d) Undiluted sample for location 2. (e) Sample diluted 1:10 for location 2. (f) Sample diluted 1:100 for location 2.

TABLE 1

Suggestion for recording results for the Ames test performed on samples from recreational and industrialized areas

Plate	CFU
Negative control	
Positive control	
Recreational area 1-1	
Recreational area 1-2	
Recreational area 1-3	
Recreational area 2-1	
Recreational area 2-2	
Recreational area 2-3	
Industrialized area 1, undiluted	
Industrialized area 1, diluted 1:10	
Industrialized area 1, diluted 1:100	
Industrialized area 2, undiluted	
Industrialized area 2, diluted 1:10	
Industrialized area 2, diluted 1:100	

CONCLUSIONS

In addition to its use in detecting the potential mutagenicity of different samples we have found the Ames test to be extremely useful for conveying key concepts to students, as well as developing problem-solving skills. Furthermore, the potential of this test as a starting point for problem-based learning is remarkable. Knowing that many colleagues were impeded from accessing their laboratory classrooms due to the COVID-19 pandemic, we decided to share data we had collected previously so that instructors could “conduct” the Ames test. For instructors who are in conditions to perform the test, we suggest they use the results we share in this resource to broaden their classroom discussions. The Ames test, with or without bacteria, is an excellent way for students to understand abstract concepts that could otherwise be difficult to visualize, while at the same

TABLE 2

Results for the Ames test performed on water from a river

Plate	CFU
Negative control	146
Positive control	1,840
River water 1	2,200
River water 2	2,150
River water 3	2,180

time allowing them to envision real-world scenarios and their possible explanations.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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We declare no conflicts of interest.

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