Science in School
The European journal for science teachers

ISSUE 61 – February 2023 **Topics** Biology | Chemistry | Engineering | Health | Science and society

Much ado about nothing: spot misleading science claims and explore rapid antigen tests and buffers

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Fighting fake facts: When a Covid test shows a positive result with cola, does testing make sense? To answer this, one must understand how antigen tests and buffers work.

A major challenge of science teaching must be to raise awareness of the importance of critical thinking. Therefore, the difference between scientific-approved facts and subjective claims, which are biased and a matter of personal belief, should be made explicit. With these activities, students can broaden their skills of thinking critically.

A further discussion about the necessity and ways to check so-called facts in general can follow. Additionally, curricular content like the function of buffers, pH levels, the structures of proteins, and the function of antibodies can be approached. This work was tested with pupils aged 16 in a science lab course offered by the university for schools. It may be used for students aged 14–19 with small alterations. To reduce costs for the COVID-19 test kits, it is recommended for students to work in groups for the activities requiring an antigen-based rapid diagnostic test (Ag-RDT).

Activity 1: Fake facts in the Austrian parliament?

In December 2020, a member of the Austrian right-wing party Freiheitliche Partei Österreichs (FPÖ) used his speech time in parliament to demonstrate that a COVID-19 Ag-RDT showed an allegedly positive result with a cola drink. He used this false-positive test to deny the efficacy of coronavirus testing in general. We can use the YouTube video of the speech[1] as an authentic and relevant anchoring phenomenon to teach the chemical principle of buffers.
This activity can be done in about 15 mins although more time might be needed for the discussion.

Materials

- YouTube video with the claim that COVID antigen tests are useless[2]
- English transcript of the video
- 1 small glass of cola drink
- 1 disposable pipette
- 1 COVID-19 Ag-RDT that delivers false-positive results with cola drink – save the buffer solution for Activity 3

NOTE: When developing this unit, we found that not all tests showed a false-positive result, possibly because some have the buffer already in the sample pad. It is thus important to try different tests before the lesson and choose one that does give a false-positive result. If you can't find any COVID antigen tests that show this effect, other antigen tests like some pregnancy tests may also work, although care must be taken in suggesting to students that they can 'cheat' some tests in this way. If no suitable tests can be found, the first two activities can still be run using the video and model, but it is much more engaging if students can test for themselves.

Procedure

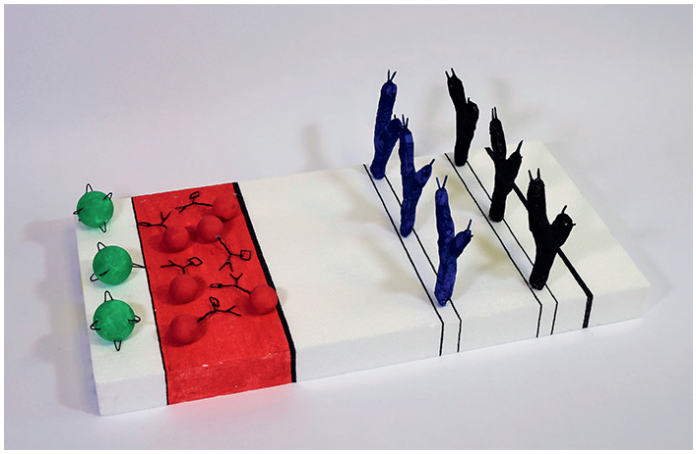
1. Show the YouTube video (German) and/or read the English transcript of the video of the politician's talk. It might be helpful to describe the way in which the politician presents his speech when only reading the transcript. You can additionally discuss local media examples of this effect, for example, of school students using soft drinks to get positive tests to stay home from school.
2. Discuss this incident with the students, collecting the reactions and beliefs of the students. Ask whether this result means that the tests are useless. The necessity to test this claim should come up.
3. Recommended: Give the students a test strip (no buffer), the soft drink, and a pipette, and have them reproduce the politician's testing procedure as exactly as possible. In the video, you can see that he drops cola directly onto the input well of the test strip with a pipette. (Reduce the number of tests needed by working in groups or a use camera to show testing.)
4. Ask students how we can explain this result. Gather ideas and discuss how they could be tested. The following questions can be used to guide the discussion:
   - How does the politician's test differ from the test instructions?
   - What is missing? Answer: the liquid supplied for applying the sample to the test.
   - What is in this liquid?

Discussion

If students have prior knowledge about the function of Ag-RDTs, they may speculate that cola drinks could change the antigens or antibodies, or that some content of the soft drink could react with the gold complexes. To be able to reflect critically, firstly, the functioning of an antigen test must be understood, and secondly, that of buffers.

Activity 2: How do antigen rapid tests work, and what are the reasons for false positives?

While the test from Activity 1 is running, the function of Ag-RDTs is explained. The activity takes about 10 mins.



Model of an Ag-RDT
Image courtesy of the authors

Materials

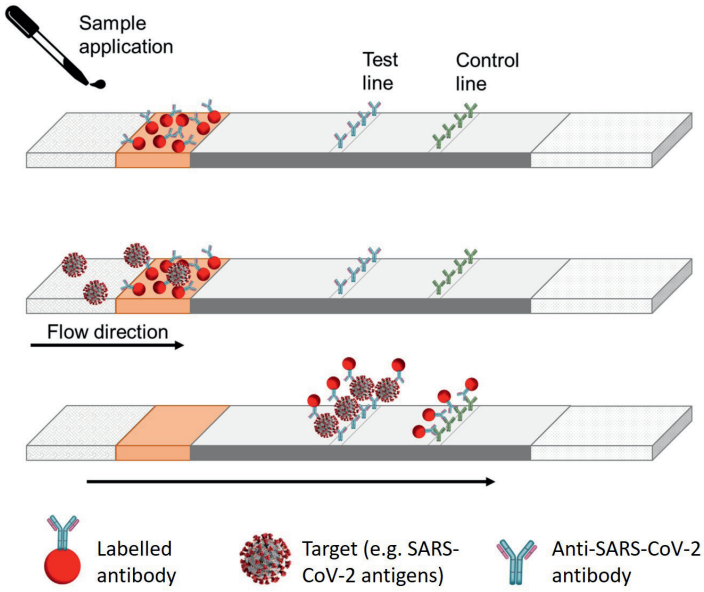
- Large-scale model of Ag-RDTs to be constructed before the lesson; see the test model building instructions in the supporting material
- Test model demonstration sheet for using the model
- Labelling cards
- An opened COVID-19 Ag-RDT (use pliers to open)

Procedure

1. Students are given a brief explanation of antibody–antigen binding (lock-and-key principle) in immunological reactions.
2. The opened test strip is shown, and ideas are collected about how such a test could work.
3. With the help of a hands-on model and labelling cards, students explore the function of an Ag-RDT (as shown on the demonstration sheet). They reproduce the different steps; articulate the idea of an antigen–antibody reaction; and understand that the test line can only show when virus antigens are present, to mediate the contact between the colloidal gold complex and the antibodies of the test line.[3]

Explanation: Function of antigen rapid tests (lateral-flow method)

The Ag-RDTs follow the lateral-flow method and are based on the principle of immune complexes, where an antigen and its specific antibody build an antigen–antibody complex.[4] The method can be used to detect COVID-19, to diagnose a pregnancy, or to predict the time of ovulation. Furthermore, these tests can also be used in nonmedical fields, for example, food and beverage manufacturing or environmental remediation.[5] In the following, the principle of an Ag-RDT is described by using the example of a COVID-19 Ag-RDT.



Scheme of molecular processes in positive COVID-19 testing[3]
©Lateral Flows, *used with kind permission*

How does the test work?

- When a test is conducted, the sample is added to the sample pad. It serves as a preliminary filter to eliminate pollutants from the applied sample. In some tests, the sample pad already contains a buffer solution that can be released to achieve the ideal pH level of the immunological reaction. In others, the sample is suspended in a buffer solution and then added to the test. This could be a reason why not all types of tests show a positive outcome when testing with cola drinks.
- The sample starts to migrate through the test, reaching the conjugate pad. It contains antibodies connected to a label, such as colloidal gold (red colour), the so-called antibody–gold complex. If the sample contains the virus, these antibodies bind to antigens (proteins on the surface the COVID-19 virus) based on the lock-and-key principle. The antibody–gold complexes (with or without bound virus) then move to the next section of the test.
- The next section is called the reaction matrix, containing the test line and control line. The test line of a COVID-19 Ag-RDT contains immobilized antibodies that also bind to antigens of the coronavirus. In the case of infection, these antibodies capture the virus particles, which are also bound to the coloured antibody–gold complex, so that a red line appears on the test.
- The control line of a COVID-19 Ag-RDT contains other immobilized antibodies that bind to the antibody–gold complex itself (whether or not it has virus attached). Since the antigen–gold complexes are used in excess, they always reach the control line, which thereupon appears red. If this is not the case, the test is invalid.
- Excess reagents move past both lines and enter the absorbent pad, which is the last zone of the test.

Activity 3: What does the buffer do?

To test the hypothesis of the missing buffer, students repeat the COVID-19 Ag-RDT with a new kit, again testing with cola, but this time adding the buffer, according to the manufacturer's instructions. While the test is running, students explore the question of what a buffer does by conducting an experiment. They use pH paper to compare the pH of the cola drink and the buffer solution.

This activity takes approximately 15 min.

Materials (per group)

- 1 small glass of cola drink
- 2 disposable pipettes
- 1 COVID-19 Ag-RDT (that delivers false-positive results with cola)
- Phosphate buffer (e.g., from the test used in Activity 2)
- 1 pH electrode
- 4 beakers
- pH indicator paper
- Student instructions handout
- Summary handout

Procedure

Part 1

1. Run the COVID-19 Ag-RDT by following the instructions, using all supplied parts of the test kit. Instead of taking a sample from the nose, use cola as the sample.

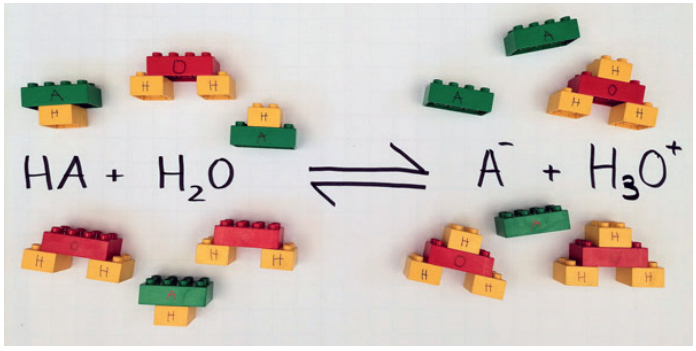
Part 2

1. While the test is running, tear off a small piece of indicator paper and use a pipette to put a drop of cola on one end of the indicator paper.
2. Take the buffer solution from the test kit and apply one drop to the other end of the indicator paper.

Part 3

1. Add 20 ml of the phosphate buffer to a beaker.
2. Measure the pH level by holding the pH electrode in the solution.
3. Take the beaker and pipette with cola drink from step 1 and slowly add it to the phosphate buffer, while continuously stirring and measuring the pH level of the solution with the pH electrode.

Optional extension: The function of buffers can be nicely explained using building bricks; see Extension activity – buffer model in the supporting material.



Model of buffer function
Image courtesy of the author

Discussion

Students should realise that the cola is acidic and that a buffer solution can stabilize the pH at a certain level.[6]

Once they compare the results of the COVID-19 Ag-RDT without (Activity 2) and with buffer solution (Activity 3), they should realize the importance of conducting a diagnostic rapid test according to the manufacturer's instructions. Analogies can be drawn to using a cake mix and adding cola instead of eggs: you can't expect things to work as advertised if you don't follow the instructions!

Depending on the students' prior knowledge of proteins, the effect of the acidic pH of cola drinks on the structure of proteins may be discussed, or even demonstrated by mixing the cola drink with (lukewarm) milk.

Students may also test further hypotheses, for example, that the acidity plays a role. Since the exact mechanism of each Ag-RDT varies, and proteins may be influenced by many factors, due to the weak interactions that determine protein folding, it is important to draw careful conclusions and take all possibilities into consideration.

Conclusion

To make a concluding statement, the students should review all previous activities. They combine their newfound knowledge to evaluate and judge the claim of the Austrian politician, which is obviously wrong. Because of the missing buffer solution during his execution, the ideal conditions (pH level) of the immunological reaction cannot be attained. It is possible that the acidic character of cola and some other drinks denatures the proteins in the tests, causing a false-positive outcome;[7] however, the exact mechanism has not been clarified. The work should be concluded by giving students the summary handout with the take-away points. The difference between beliefs or assumptions and scientifically tested and reproduceable results should be discussed. Teachers can link this what students have learned about the scientific method (e.g., dependent, independent, and control variables) and discuss how considering all the factors that affect the experimental system is important for evaluating whether the results really support the conclusion. «

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Resources

- Pölloth B, Röhrig H, Schwarzer S (2022) Why is there a red line? A high school experiment to model the role of gold nanoparticles in lateral flow assays for COVID-19 *Journal of Chemical Education* **99**: 2579–2587.
- Explore how research projects are chosen for funding with this role-playing activity: McHugh M et al. (2021) What is it good for? Basic versus applied research *Science in School* **55**.
- Produce wonderful colours using microscale methods: Worley B, Allan A (2021) Little wonder: pH experiments the microscale way *Science in School* **54**.
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