Our project meets a need!
We turned to experts in various fields (doctors, scientists, ethicists) to assess the project. They gave us insight which we integrated to improve the product, highlighted in green.

Teaching the public
Through talks, youth educational meet-ups and university open-days, we:
- raised awareness about antibiotics use
- explained synthetic biology and working principles of home-diagnostics devices
- let people experiment with printed paperstrips

Sample Processing
- Our detection limit from in vitro transcribed and in vivo extracted RNA is in the nM range. We need an amplification scheme to reach a more relevant detection.

Cas13a Characterization
- Cas13a sensitivity goes down with increasing concentration; while the kinetics of cleavage go up. We chose a compromise concentration of 10 nM Cas13a. The detection limit was less than 10 nM target RNA.

Overview
To prevent further spread of antibiotic resistance, we designed an affordable, rapid point-of-care test for infectious diseases to distinguish viral and bacterial pathogens: CascAID. The module of this device enable extraction, amplification and detection of target RNA sequences by fulfilling the ASSURED criteria.

Cas13a fighting the antibiotic crisis
Cas13a is a CRISPR-associated protein which recognizes RNA sequences, with the help of a complementary CRISPR RNA (crRNA). It then becomes an unspecific RNase. Its single-nucleotide specificity makes it ideal for differentiating bacteria and viruses, by recognizing a unique 28-nucleotides sequence.

Antibiotic resistance crisis: a major health threat
Because antibiotics are prescribed unnecessarily, bacteria grow resistant and simple infections are on the rise.

References
Pawde K. et al., Cell (2016).
Carrillo E. et al., Analytical Chemistry (2009).

Who did the work
- Doris, J. Chacin, E.
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- Sherpa, D.
- Neumayer, C.
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Results
We successfully used Cas13a in a fluorescence-based assay on paper to distinguish viral and bacterial at RNAs with a detection limit of 10 nM. Based on the most affordable and sensitive fluorescence detection in iGEM, to our knowledge.

Costs
- liters of reagents: $0.005
- single-use paper strip (no enzymes & chemicals): $0.20
- reagent mix: $0.05
- energy supply: $0.006
- labrent: $0.005

Detector
- We measured a time trace of RNase Alert cleavage by Cas13a (blue). Positive control is a RNA displacement (blue A), negative control is only RNase Alert.

4 alternative readouts
- Functional AuNPs, Spinach-Aptamer
- Easy to interpret

Readout
- The spinach-aptamer increases the fluorescence signal upon binding. As directed Cas13a is then detected by a decrease in fluorescence, when the RNA binding site is cleaved.

Gold nanoparticles (AuNP) allow for a colorimetric readout on paper. RNA-cleaved AuNPs are dispersed by RNase activity, leading to a shift in absorbance, and a visible signal. We show that our method works with RNases as a proof of principle.

Read
- We designed additional colorimetric readouts that include an amplification step, increasing the detection sensitivity.

References
Pawde K. et al., Cell (2016).
Carrillo E. et al., Analytical Chemistry (2009).