‘From women for women’: A citizen science approach engaging women in the isolation and application of the vaginal health-associated bacterium *Lactobacillus crispatus*

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Abstract

A vaginal microbiome rich in *Lactobacillus crispatus* is associated with good reproductive and sexual health outcomes. Dysbiosis, indicated by a loss of *Lactobacillus crispatus,* is a risk factor for urogenital infections such as the clinical diagnosis of bacterial vaginosis (BV) or urinary tract infections. While many scientists explore probiotics using a conventional pharmaceutical approach, concerns about the accessibility and affordability prompt an investigation into a preventive approach using this naturally occurring bacterium. Our study is aimed at the exploration of a potential woman-friendly vaginal probiotic product by use of the naturally occurring bacterium *Lactobacillus crispatus*. Citizen scientists actively participated in a two-day practicum, successfully performing procedures with self-collected vaginal swabs. The practicum received a positive response from participants, who demonstrated notable engagement and enthusiasm. With expert guidance, participants without a laboratory background were able to successfully execute the assigned tasks. From the Dutch *crispatus* Citizen Science Collective of 48 women, 22 succeeded to isolate their own *Lactobacillus crispatus* strains, using a Loop-Mediated Isothermal Amplification (LAMP) protocol for identification. In addition, 48 meta genomes and 54 whole genomes from 22 individuals were sequenced for comparative analysis by an external company. This project effectively engaged a community of women into isolation of *Lactobacillus crispatus* strains from their vaginal microbiota followed by *in vitro* characterization experiments and a hackathon for the development of a probiotic product. Our citizen science approach opens up collaboration possibilities and new avenues for exploration in vaginal health, facilitating community involvement and the development of targeted intervention to enhance women’s well-being.

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Keywords
Vaginal health, probiotic, *Lactobacillus crispatus*, citizen science

Before start

This protocol can be followed in a low-resource environment. All volunteers participating in the initiative must sign a consent form, ensuring they are both willing and legally competent to understand the implications of their involvement. It is paramount that privacy is upheld and guaranteed at all times, safeguarding the confidentiality of those involved.

Setting up a women's collective

The ideas for the citizen science initiative described here were presented to "The *crispatus* foundation" to manifest its vision and curate a collection of *L. crispatus* isolates. Participants contribute their isolates under a licensing agreement, allowing the foundation to negotiate sub-licensing for probiotic product development. (Supplemental file 1: Appendix A. Licensing agreement).

Participants retain ownership of their contributions, ensuring their intellectual property rights are safeguarded. Donors, upon joining a panel, gain oversight and voting rights concerning sub-licensing agreements. They are entitled to receive 20% of the profits generated, while the remaining 80% of the foundation's profits are dedicated to funding new research initiatives.

For privacy concerns, every participant chose their own pseudonym. Zivver|(Zivver, Amsterdam, The Netherlands), a platform that allows the exchange of information in an easy and secure way, was used for sharing sensitive information during digital communication.

Recruitment

To keep the sample size closer to our target number, recruitment was mostly based on word of mouth, newsletters, a small publication in a local newspaper or a flyer on a college campus. Initially, we attracted a diverse group of women interested in participating in the study, all with different backgrounds and life experiences spanning ages 18 to 75. As we progressed, however, challenges in collecting *L. crispatus* from women aged 40 and above led us to refine the criteria, focusing on women between the ages of 18 and 40. We had a total of five practicals with 48 participants. We were able to isolate *L. crispatus* from 22 women. The practicals were held at three different locations in The Netherlands: Vrije Universiteit Amsterdam, Avans Hogeschool, Breda and BioARTLaboratories Eindhoven.

Practicals

We organised a two-day practical with two hour sessions each day. Upon registration, we shared additional information with potential donors about the importance and the reason for our project (Supplemental file 2: Appendix B. Participant information). Participants received an online questionnaire (Supplemental file 3: Appendix C. Participation in the *crispatus* Study - Questionnaire), that focused on hormonal aspects, and a sampling kit at their home address containing:

- 2 sample collection tubes. One for metagenomic sampling (with DNA/RNA shield buffer), and one for microscopy and isolation (e-swab with Amies transport medium).
- A swab for a pH self-test.
- An information booklet that also served as a personal lab journal.

The contents of the sampling kit that a donor would receive are illustrated in Figure 1.

The women were advised to take the swabs a maximum of four hours before the start of the practical (Supplemental file 4: Appendix D. Section on Swab Sampling from the Lab Journal). Taking the swabs on location was also an option. We used a lactation room or bathrooms for this purpose. A short introduction was given about the purpose of the project and how we go about

achieving our objective. Since the practical was taking place in a laboratory, basic safety lab instructions were given to the women.

The informed consent and licensing agreement were discussed and signed before starting the practical (Supplemental file 5: Appendix E. Consent form *crispatus* practical). The women were also informed that they could stop research participation at any time without having to give any reason for it.

Figure 1 Content of a sampling kit, containing i) one collection tube with DNA/RNA shield buffer for metagenomic sampling; ii) one collection tube (e-swab) with Amies transport medium for isolation and microscopy; iii) a swab for a pH self-test; and iv) an information booklet that also served as a personal lab journal. Picture created by Rosanne Hertzberger, 2023.

Microscopy

Citizen scientists fixed and stained a droplet from the e-swab collection tube on a microscope slide and were assisted to visualise their vaginal sample at 1000x magnification. Epithelial cells and lactobacilli were easily identified. Participants made descriptions of their samples in their lab journal and received a printed screenshot, as illustrated in Figure 2. See Supplemental file 6: Appendix F. Section on Microscopy from the Lab Journal, for the instructions provided to the donors.

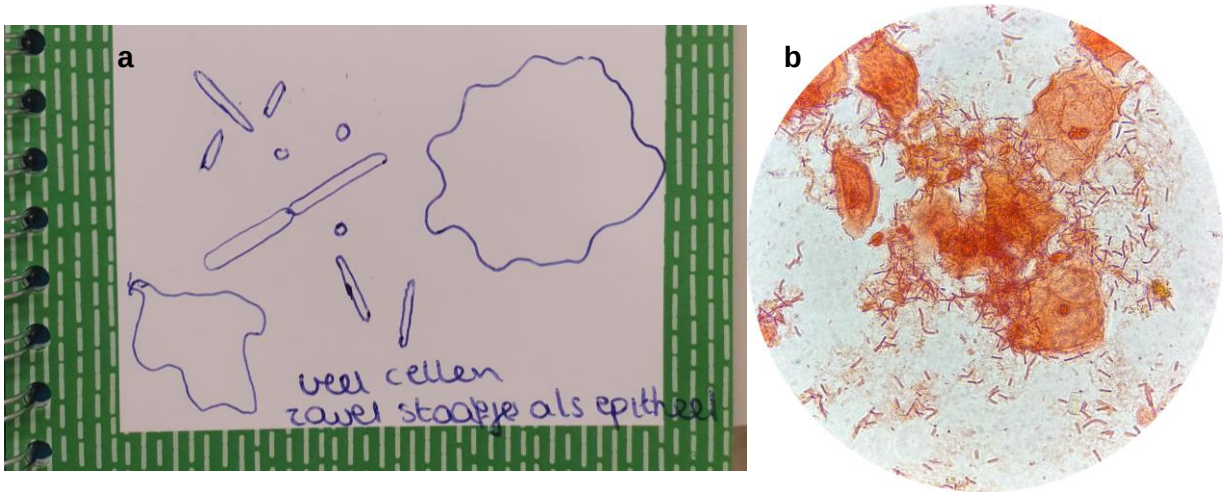

Figure 2 a) Illustrated description of microscopic view in a lab journal. b) Microscopic picture of vaginal sample showing large vaginal epithelial cells with a cell nucleus and small rod-shaped *Lactobacillus* bacteria.

Lactobacillus crispatus colony identification using a colorimetric test

For the identification of *L. crispatus* colonies we needed to implement a microbial molecular technique that would be easy for the general public to carry out without the need for a microbiology training. We used a *L. crispatus*-specific Loop-mediated isothermal amplification protocol (LAMP) as an alternative to colony PCR to allow participants to identify strains of *L. crispatus* [1]. See Supplemental file 7: Appendix G. Section on Identification and Isolation from the Lab Journal, for the instructions provided to the donors and Supplemental file 8: Appendix H. Loop-mediated isothermal amplification protocol, for the protocol.

The LAMP is a DNA amplification method with high specificity and amplification efficiency. It allows the reaction to take place at a constant temperature, 65˚C, within 30 minutes. It uses 4 primers and a DNA polymerase with strand displacement activity. The WarmStart® Colorimetric Master Mix (New England Biolabs, Ipswich, Massachusetts, United States) that is used in this protocol, has a low Tris buffer concentration at pH 8. The mix contains a pH indicator, phenol red, that changes

colour from red to yellow below a pH of 6,8, as shown in Figure 3 [2]. This colour change occurs

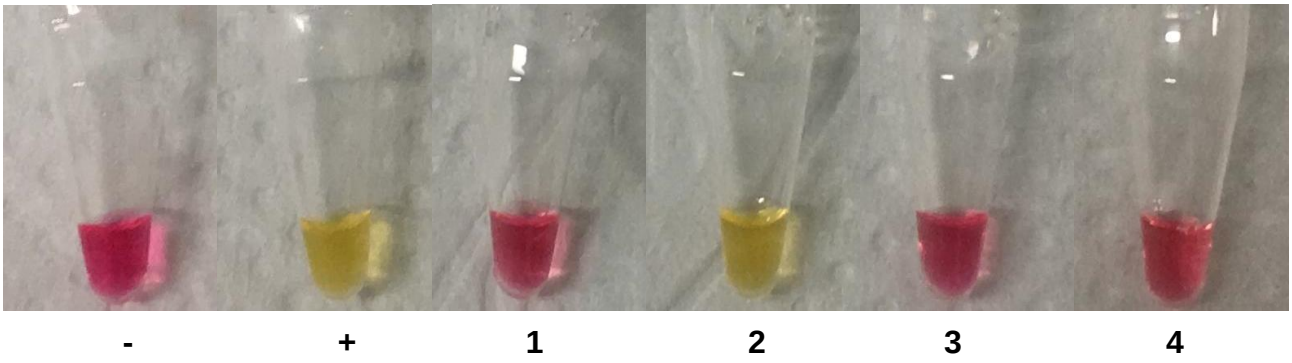

Figure 3 Validation results of the Loop-mediated isothermal amplification method test for the citizen science practica. **-** negative controle, + positive controle, 1 ***L. plantarum***, 2 ***L. crispatus,*** 3 ***L. delbruecki*** subsp. ***bulgaricus***, 4 ***L. gasseri***

due to a drop in pH by protons that are released during the amplification process [3].

A previous study designed unique LAMP primers for *L. crispatus* and showed that the LAMP test has a detection limit of 10 fg DNA [1]. This was followed up by a dilution step in the protocol with 100 µL Milli-Q to dilute any lactic acid associated with the colony and prevent false positives.

The LAMP test was validated using colonies of *Lactobacillus plantarum, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus gasseri* and *Lactobacillus crispatus* as illustrated in Figure 3. Furthermore, we tested 134 colonies of vaginal isolates obtained during the practical and compared the outcome of the LAMP test with a colony PCR using *L. crispatus*-specific primers [4]. A total of 61 colonies showed a 145 bp band in the colony PCR analysis, matching 100% with the positive outcome of the LAMP test.

Isolation and cultivation

For *L. crispatus* isolation we used Tryptic Soy Broth (TSB) agar plates supplemented with 10% horse serum, 1g/L tween 80 and 10g/L glucose at pH 5 adjusted with 10% acetic acid as previously described [5].

To ensure that each participant could successfully isolate *L. crispatus* colonies, despite substantial variation in bacterial load, each citizen scientist inoculated two TSB plates. The first plate received a large droplet application via a Pasteur Pipet, using L-spreaders for the spread plate method. The second plate was inoculated with a small droplet using an inoculating loop for the streak plate method (See Supplemental file 9: Appendix I. Section on Sample Plating from the Lab Journal, for

the instructions provided to the donors). Figure 4 illustrates the difference in bacterial growth

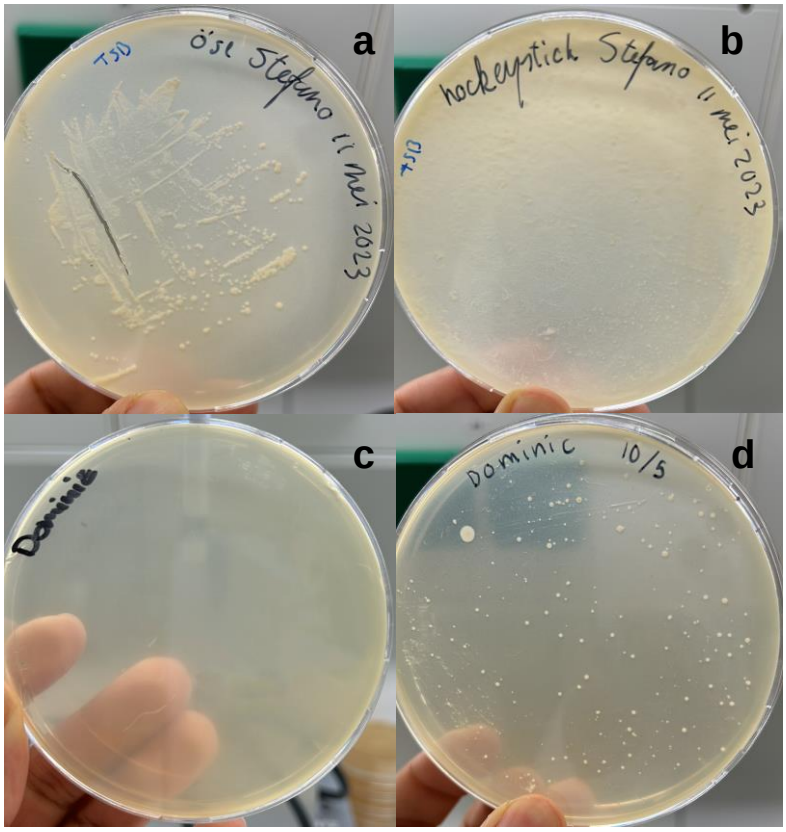

Figure 4: Variation in bacterial growth between vaginal swabs of two citizen scientists on Tryptic Soy Agar.
a) and c) were inoculated using a loop.
b) and d) were inoculated using an L-spreader.

between two citizen scientists on Tryptic Soy Agar.

The plates were incubated anaerobically at 37℃ for 48-72 hours using Oxoid™ AnaeroGen (Thermo Fischer Scientific, Waltham, Massachusetts, USA) system. We selected the colonies based on colony morphology which included being round, creamy white, with a raised central area, and having a slightly rough texture with irregular edges. Additionally, we used reference plates showing the morphology of *L. crispatus* along with other known lactobacilli including *L. gasseri, L. plantarum* and *L. delbruecki* subsp. *bulgaricus*. Later on, we confirmed the identity of the selected colonies with the LAMP test. Positive colonies were restreaked on MRS plates. Alongside choosing their own pseudonym, participants were also able to name their *L. crispatus* isolate.

References

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Supplemental Material

Supplemental File 1: Appendix A — Licensing agreement

License Agreement Concerning the Exclusive Use of Isolated *Lactobacillus crispatus* Bacteria and Derived ("Cloned") *Lactobacillus crispatus* Bacteria.

Parties

, residing at ,

available at hereinafter referred to as 'licensor';

And

Stichting *crispatus**, Chamber of Commerce number 78105323, represented by Rosanne Yente Hertzberger, reachable via info@crispatus.org, hereinafter referred to as 'licensee' (see articles of association in annex 1).

Whereas the following is considered:

1. Licensor has the right to use all *crispatus* bacteria donated by licensor as described in annex 1 to this agreement, hereinafter referred to as 'the licensed';
2. Licensor, as a citizen scientist, will participate in the "From Women for Women: Share Your Health with Your Own Vaginal Bacteria" research conducted by licensee on distinctive characteristics of *crispatus* bacteria;
3. Licensee may include the *crispatus* bacteria that licensor would like to include in a collection for granting third parties access through sublicenses as part of this research;
4. These bacteria will become part of a mixture of *crispatus* strains, and this mixture will become part of a product aimed at promoting health;
5. It is important for licensee to obtain exclusive user rights over *crispatus* bacteria;
6. Parties consider it important that licensor retains influence over the *crispatus* bacteria she licenses;
7. To this end, licensee will establish a donor panel, of which licensor may become a member.

Agreed upon:

Article 1 - Description of the Licensed:

This agreement is concluded on the rights of use for all *Lactobacillus crispatus* strains that licensor cultivated, selected, and isolated as part of the "From Women for Women: *Lactobacillus crispatus* Isolation" practical. Genetically modified (cloned) derivative strains of the original isolates also fall under this license.

Article 2 - Description of the License:

1. Licensor grants to licensee the exclusive right to all usage rights that may exist in or arise from the use of the licensed. This includes all products, formulations, extracts, applications, preparations, and other possible methods of use (hereinafter referred to as 'use') worldwide (hereinafter referred to as 'the territory'), which licensee accepts. Licensor will refrain from using or transferring her *crispatus* strains to other parties and will not withdraw any of the strains isolated by her from this agreement.
2. Licensor agrees to the use of one or more isolated *crispatus* strains for 'standard research framework', which includes: the cultivation and use of the strains for research and development purposes of the ZonMW project From Women for Women and for non-commercial scientific research.
3. Licensor has the opportunity to actively participate in the donor panel (board decision and house rules in annex 1) for the use of strains from the *crispatus* bacteria collection outside the standard research framework.

4. Licensee is exclusively and unconditionally authorised to grant sublicenses subject to the approval of the donor panel according to the house rules of the donor panel. The agreements regarding the donor panel and the articles of association of Stichting *crispatus* are attached as Annex.
5. Licensor grants licensee the right to all possible intellectual property rights or copyright arising from the research or use of the *crispatus* bacteria, and licensee acquires the rights to improvements/changes made by the licensee.
6. Any proceeds from the sublicense agreement are owned by Stichting *crispatus* and will be used for research and development, in line with the character and objectives of the Foundation.

Article 3 – Duration of the Agreement

1. The license agreement is valid for 30 years and commences on [date of signing].
2. Each of the parties is entitled to terminate this agreement with immediate effect in the event of:

a) Dissolution of Stichting *crispatus*; upon dissolution, the license agreement is automatically terminated, as well as any sublicense agreements.
b) Improper management as referred to in Article 2:9 of the Civil Code. The agreement shall be considered terminated when a court decision becomes irrevocable.
c) Inactivity for more than 2 years regarding use in research, development, application, including commercial activities.

Article 4 - Compensation

1. Licensee will annually allocate an amount to the collective of licensors as compensation for the right granted in Article 1. This amount consists of 20% of the total revenue (defined as income resulting from the use of one or more strains minus the costs incurred by licensee) of the licensed product, arising from sublicenses approved by the donor panel, as well as other sources of income related to the strain(s).
2. This amount will be distributed proportionally among all individual licensors, regardless of the number of strains included in this license agreement or the value, effect, or other properties of these strains for sublicense. The amount will also be distributed to participants of the *crispatus* practice as organised in 2023, including 1) citizen scientists who are not actively participating in the panel, 2) citizen scientists who did not isolate a *crispatus* bacterium, and 3) citizen scientists whose isolated strain is not part of any used selection of strains in the sublicense from which revenues are generated. This compensation will be paid as long as this license agreement is in force, which is 30 years, unless otherwise agreed upon in new sublicenses.
3. Licensee will provide an annual statement of the income received and the costs incurred in the preceding year, as well as a breakdown of the amounts owed by licensee to licensor.
4. Licensee will use the remaining proceeds to fund research to further develop the collection, explore new applications for the collection of bacteria, and other research aimed at promoting women's health.

Article 5 – Guarantees and Obligations

1. Licensor guarantees to be the rightful owner of the *crispatus* strain(s) and is authorised to enter into this agreement.
2. No other licenses or any other right or claim of a third party encumber the rights granted in the license. Licensor shall indemnify licensee against all claims in this regard and shall reimburse licensee for all costs and damages resulting from such claims; the costs borne by licensor shall not exceed those of the license income received by licensor.
3. Parties shall immediately inform each other if they observe any infringements of the usage rights.

Article 6 - Termination of the Agreement

1. The agreement is valid for 30 years and is, according to the intention of both parties, non-terminable.
2. Parties may only terminate this agreement if there is a sufficiently weighty ground, as described in Article 2, sub-paragraphs a-c.

3. If one of the parties wishes to terminate the agreement when there is no weighty ground as referred to in Art. 2 sub-paragraphs a to c, the terminating party shall be required to pay compensation to the other party, which will be determined by independent parties/mediation.
4. In case of a conflict between licensor and licensee, they shall undergo independent mediation, whereby both parties shall appoint an arbitrator who, in consultation, will choose a third independent mediator to resolve the conflict.
5. Termination must be done by registered letter, and in the case of Article 2 sub-paragraphs a or b not being applicable, the notice period shall be one year.

Article 7 – Consequences of Nullity or Annulment
If a part of this agreement is declared null or void, it does not affect the other provisions in the agreement. A provision that is null or void will be replaced in that case by a provision that comes as close as possible to what the parties had in mind when concluding the agreement on that point.

Article 8
1. Dutch law applies to this agreement.
2. In case of any disputes, the court in the district where the registered office of the Foundation is located shall have jurisdiction. This is currently Amsterdam.
3. Parties will send messages to each other to the email or physical address as stated at the beginning of the contract. If the address of one of the parties changes, they will notify the other party of the new address. Until this happens, it cannot be held against the other party if digital or physical mail is sent to the old address.

Article 9 – Licensee and Licensor Reserve the Right to Modify the Contract

CITIZEN SCIENTIST / DONOR
NAME

SIGNATURE
PLACE
DATE

ON BEHALF OF STICHTING *crispatus*
NAME

SIGNATURE
POSITION
PLACE
DATE

Annex 1: Household Regulations of the Donor Panel of Stichting *crispatus*

Article 1.1 Powers of the Board and the Chairperson:
The Board:
a. Governs and represents Stichting *crispatus*.
b. Makes board decisions.
c. May establish working groups for a specific period. Such a group consists of at least one board member, and non-board members may also be part of the group.
d. Decides individually on expenditures up to 1200 euros in ad hoc situations.
e. Negotiates with parties regarding the terms of a sublicense for the use of the strain collection.
f. Drafts a concept sublicense to be submitted to the donor panel.
g. Sends the concept usage agreement to the Chairperson at least 28 days before the vote.
h. Has the opportunity to seek advice from the donor panel on new research projects.

i. Recognises the importance of good communication with:
    i. Donors
    ii. Partners
    iii. Sublicensees
j. Periodically keeps them (i-iii) informed through her preferred communication channels.

The Chairperson:
a. Assumes general leadership of the foundation.
b. Represents the foundation externally.
c. Consults with official authorities.
d. Leads the board.
e. Serves as the primary point of contact for board members.
f. Collaborates with other board members to set the agenda for each meeting.
g. Chairs the board meetings and the annual meeting.
h. Ensures that decisions are made in accordance with the law, the articles of association, and these household regulations.
i. Coordinates and directs activities.
j. Ensures that board members fulfil their duties properly and addresses them if this does not seem to be the case.

Article 2.1
The Donor:
a. Is authorised to attend meetings of the donor panel.
b. Is authorised to cast one vote per person during the meeting, regardless of the number of donated *crispatus* strains.
c. Is authorised to propose other suggestions to the board of Stichting *crispatus*.

The Donor Panel:
a. Exclusively consists of citizen scientists:
iv. Whose own crispatus bacteria have been isolated.
v. And who have signed a license agreement placing their exclusive usage rights under Stichting *crispatus*.
vi. Who participated in the first five bacteria collection practices in 2023 but did not isolate a crispatus bacterium.
b. Operates collectively, with each member having an equal vote, regardless of the number or value of the donated strains in the collection.
c. Collectively has the authority, with a majority vote, to provide binding advice to the board of Stichting *crispatus* (licensee) regarding the acceptance or rejection of a sublicense agreement drafted by the board for the use of one or more *crispatus* strains, outside the usual research framework, for example, for research or commercial purposes of third parties.
d. Collectively provides advice on new research projects with a majority vote.
e. Appoints the chairperson of the donor panel for a term of three years.
f. Extends the term of the chairperson appointed by them with a majority vote.
g. Empowers, in the absence of a vote, either in person or through a designated representative, another member of the donor panel excluding a board member of Stichting *crispatus* as the representative.

The Chairperson:
a. Is responsible for leading the meeting.
b. Is appointed for a term of three years, which can be extended with the consent of the donor panel.
c. Can be dismissed at any time by the donor panel, after which a vote follows to select a new Chairperson.
d. Joins the board of Stichting *crispatus*.
e. On the initiative of the board, calls a meeting of all donors to provide advice on the sublicense agreement drafted by the board of Stichting *crispatus*.

f. On the initiative of the donor panel, calls a meeting of all donors to provide advice on matters where a vote can reasonably be expected.
g. Is responsible for distributing and ensuring all donors are informed about the draft license agreement.
h. Actively endeavours to notify all donors of this meeting in all reasonable ways.
i. Determines the time and place for the donor meeting, ensuring that all (or as many as possible) donors can attend.
j. In her absence, she appoints another donor to lead the meeting, or this is arranged by the panel itself during the meeting.
k. Casts the deciding vote if there is no majority in a vote because the result is 50/50.

The Donor Meeting:
a. Appoints the chairperson from among the donors.
b. Takes place digitally or in person.
c. Is announced by (i) the board of Stichting *crispatus*, or (ii) the chairperson of the donor panel, or when (iii) 20% of the total donors request it from the chairperson.
d. The donor panel provides during the meeting:
    i. Binding advice on (sublicense) proposals from the board of Stichting *crispatus*.
    ii. And/or advisory advice on all other matters.

Article 2.2
Voting of the Donor Panel:
a. Takes place during the meeting unless otherwise agreed with either the board or the chairperson.
b. Is purely collective, where all donors individually cast their votes but can only make a decision by way of a majority (half of the votes cast +1).
c. Is limited to the collection of bacteria that is licensed under Stichting *crispatus* at the time of the vote.
d. Occurs regardless of whether the donated strain from a donor is part of that sublicense.
e. Can only reject a proposal from the Foundation Board with a majority vote (half of the votes cast +1).
f. In the event of a '50 for, 50 against' result, it will be announced again within a timeframe agreed upon by the panel, after which another vote will be held.
g. In a second, equal vote (50 for, 50 against), the chairperson's deciding vote will be determined.

*Stichting *crispatus* is the official name of the foundation. In the main text, Stichting *crispatus* is referred to as the *crispatus* foundation.

Supplemental File 2: Appendix B — Participant information

Hi,
you've expressed interest in participating in our project about vaginal bacteria. We're very pleased about that!

As a citizen scientist, you can help us understand and ultimately improve our vaginal health. To do so, we'll work together in the lab.

Please read the information in this document carefully to understand what participation entails.

Best regards,

Rosanne Hertzberger

You will receive at home:

- a collection kit with swabs and a pH test;
- a personal lab journal with information and space for your findings
- a link to an online questionnaire, where we ask about your background, any contraception, menopause, and menstrual cycle.

Date and Location:
You have chosen the practical with a first part on [**Date**] from [**Time**] the second part on [**Date**] from [**Time**]. The location is [**Address**]. This is located [**Location description**] If you are running late or if there is anything else, call or text Rosanne at [**phone number**].

Summary and Practical Information
On this page, you will find a concise overview of the most important information.

Why this information?
We want to ensure that you are well-informed about the study. This way, you will know exactly what is expected of you, what we will be doing in this research, and why. You will also have a clear understanding of what you are giving consent for.

1. Introducing the *crispatus* Team

Researchers Rosanne Hertzberger and Shardelice Illidge from Vrije Universiteit Amsterdam will guide you through this research.

In this project, Vrije Universiteit Amsterdam collaborates with:

- *crispatus* Foundation: responsible for further development of the collected bacteria and for your involvement. It is a non-profit foundation.
- Yoni: this company develops innovative products for feminine hygiene, such as new types of tampons, also known as 'femcare'.
- Winclove probiotics: this company develops bacteria that improve our health, known as probiotics.
- Baseclear: this company will assist in mapping the DNA of the collected bacteria and the flora in the samples.
- The research is funded by in-kind contributions from the above partners, by subsidy from HealthHolland/ZonMw, and by contributions from the Foundation For Women By Women and the Equileap Foundation.
- With contributions from Daniella Gidaly, Elske Wits, Anne Vogel, Eva van Rossum, Heleen Eising, Michelle Haak, and many other volunteers and citizen scientists. Many thanks!

2. Why this research?

In your body, there are bacteria, for example, in your intestines and in your vagina. *Crispatus* bacteria (formally known as *Lactobacillus crispatus*) are primarily found in the vagina. These bacteria make the vagina very acidic: they are lactic acid bacteria. We believe they may help prevent infections.
The main goal of this practical is to learn more about the bacteria in the vaginal flora, especially the small differences between the *crispatus* bacteria. Additionally, we want to collect *crispatus* bacteria to investigate in the future whether they can also colonise the vagina of other women who do not have this bacteria. It is possible that these bacteria may help other women, for example, to prevent infections or to restore the flora after an antibiotic treatment, but this is still unclear.

3. What does participation entail?

Doing the lab work yourself

Citizen Science

This is a citizen science project, which means you will be conducting the research together with the researchers. This way, you will learn a lot about the bacteria and other microorganisms in the vagina. In the future, we will further study the bacteria that we cultivate together during this practical. If you wish, you can remain involved in the research to learn about the results and whether the collected bacteria can actually be utilised for femcare products.

Practical

In the hours preceding the practical, we ask you to collect two vaginal samples using swabs. We will use these during the practical for research. This is not difficult to do on your own. Additionally, we ask you to determine your vaginal acidity (pH) at home. This is also easy to do by yourself.
The practical itself takes two sessions of two hours each. During the practical, you will actively perform various lab tasks under guidance.

Use for research and development of a commercial product: your voice matters

If your contribution does indeed yield *crispatus* bacteria (this will not be the case for everyone), we hope you will want to have a say in what happens with the bacteria. You will then be officially a 'donor'. For this, we invite you to the 'donor panel' of the *crispatus* Foundation. This means that you may be occasionally asked to vote on usage agreements with third parties, such as the company Yoni, who may use the bacteria in one of their products (such as tampons) in the future.

If a majority of donors do not agree to a specific use of the bacteria, it will not proceed. The panel also has an advisory function, and you can suggest research topics to the board of the Foundation. Participation in the donor panel is not mandatory.

Finally, the commercial use of the bacteria may generate income for the *crispatus* Foundation in the future. This money will be used for new research, but 20% of the proceeds will also go to the donors in total. The proceeds are the money left after deducting all costs. We do not yet know if and when these proceeds will come and how much the amount will be. The Foundation is a non-profit, and the directors are unpaid.
These agreements will be documented in a license agreement with the *crispatus* Foundation. We will go through the content of this agreement during the practical. You can choose whether you want to sign this agreement and whether you want your *crispatus* bacteria to be part of the collection.

If you no longer wish to participate or if you want to discontinue the practical

Participation in the research is entirely voluntary. You can decide to stop before, during, or after the practical, and you do not need to state why you are stopping. However, you must inform the researchers of this. In the future, you can also always choose to stop participating in the donor panel.

You can withdraw your consent for the use of your data at any time. This applies to both its use in this study and in other research. However, if researchers have already collected and used data for research when you withdraw your consent, this data will not be destroyed. Your data will no longer be used for further research and new analyses.

As for your bodily material (the samples), the researchers will destroy them if you withdraw your consent. However, if measurements have already been taken with your bodily material, the researcher may continue to use the results. Once isolated, your *crispatus* strains will remain part of the collection. If you sign the license agreement with the *crispatus* Foundation, they may also be used for future research and development. Your name and personal data will not be used in the research during and after your participation. You can read about how we handle your privacy below.

What do we do with your data and how do we protect your privacy?

During the practical, we collect, use, and store data in order to answer the questions of this research. We also plan to publish the results of the research in the future. To protect your privacy, you will choose your own pseudonym during the practical. We will use this pseudonym on all the data and material we collect during the practical. The data directly referring to you will not be used after the practical.

We will keep the file that links your pseudonym to your name in a secure location at the university. Only the researcher and members of the research team will know which pseudonym you have chosen. When we process or share your data, we will always use only your pseudonym. In reports and publications about the research, no one will be able to tell it was about you.

Your contribution is not entirely anonymous in a few instances:

- During the practical, you will work side by side with other citizen scientists (up to a maximum of 15) and some female researchers/guides from Vrije Universiteit Amsterdam. They may know which pseudonym you choose and may see your vaginal samples during the processing steps. We handle this confidentially. Only female participants and guides will be present at the practical sessions.
- To invite you to the donor panel and involve you in the proceeds, the *crispatus* Foundation will need your data. Therefore, we will share data with the *crispatus* Foundation about the number and type of *crispatus* bacteria you isolate during the practical, along with your full name. So, 'participant ..... [name] has isolated isolates ... [name bacterium] and ... [name bacterium] and ... [name bacterium].'

Your data may also be relevant for other scientific research in the field of the vaginal microbiome and women's health after this research concludes. For this reason, your data will be kept for 10 years at Vrije Universiteit Amsterdam. Again: the file that links your pseudonym to your name will be kept in a secure location at the university.

You will personally gather a portion of the research results and record them in your own lab journal. If you desire a digital copy of your other data, you may request it from the researcher.

If you want to know more about your rights regarding the processing of personal data, please visit https://www.autoriteitpersoonsgegevens.nl/nl/over-privacy/persoonsgegevens. Do you have questions about your rights? Or do you have a complaint about your privacy? Please contact functionarisgegevensbescherming@vu.nl.

You can also always contact the researchers responsible for the practical: Rosanne Hertzberger, r.y.hertzberger@vu.nl, or Remco Kort, r.kort@vu.nl (not present at the practical sessions).

Supplemental File 3: Appendix C — Participation in the *crispatus* Study - Questionnaire
Hello,

We're very pleased that you're interested in participating as a citizen scientist in the *crispatus* study on vaginal bacteria.

We'd like to know a bit more about all participants. Previous research has shown that various factors can influence vaginal flora. For instance, post-menopausal women have a different vaginal environment compared to those who still have a menstrual cycle. Hormones are therefore significant. Additionally, ethnic background and diet may also play a role.

Kindly complete this questionnaire on the day prior to the practical or on the day of the practical itself.

That's the reason for this questionnaire. Thank you in advance for sharing, and we look forward to seeing you at the practical!

Best regards,

Rosanne Hertzberger (on behalf of the entire *crispatus* team)

To begin: what is your pseudonym? This is a name you chose upon registration.

_____

What is your age group?
- Younger than 21
- 21-30 years
- 31-40 years
- 41-45 years
- 46-50 years
- 51-60 years
- 61-70 years
- 71-80 years
- Over 80 years

Were you born outside of the Netherlands?
- Yes
- No

Were both of your parents born outside of the Netherlands?
- Yes
- No

As ethnicity plays a role in vaginal flora, we'd like to know a bit about your ethnic background. Therefore, the following question: do you identify with any of the following groups? (multiple answers possible)
- Dutch
- Moroccan
- Turkish
- Hindustani-Surinamese
- Afro-Surinamese
- Antillean
- Chinese
- African
- Other, namely_____

Because hormones, menstrual cycle, menopause, and previous childbirths play a role in vaginal flora, the following questions are relevant: Do you have biological children and if so, how was the method of delivery? (multiple answers possible)
- Vaginal birth
- Cesarean section
- No biological children.

Do you menstruate? If so, is it regular or irregular? Regular, for example, would be every 25-30 days.
- I menstruate regularly.
- I menstruate irregularly.
- I do not menstruate.
- Other, namely:_____

In case of menstruation: what was the first day of your last menstruation? If you don't know or it's not applicable, you can leave this blank.

_____

In case of irregular or absence of menstruation, what is the cause? (multiple answers possible)
- I am pregnant.
- I am breastfeeding.
- I am using contraception.
- Age (menopause).
- Unknown.
- Not applicable.
- Other, namely:_____

In the case of absence of menstruation due to menopause: has your last menstruation been longer than 1 year ago?
- Yes
- No
- Not applicable

Are you using hormone therapy for menopause-related symptoms?
- Yes
- No

Do you use any of the following contraception methods?
- Birth control pill
- Hormonal intrauterine device (IUD)
- Copper intrauterine device (IUD)
- I do not use contraception myself.
- Other, namely:_____

Because diet may potentially be related to the vaginal microbiome, we ask the following: how often do you eat meat on average per week?
- Every day
- At least one day
- Less than one day per week
- Never

How often do you eat fish per week?
- Every day
- At least one day
- Less than one day per week

- Never

This was the last question. Once again, thank you very much for sharing your information and for your participation. We look forward to seeing you at the practical.

Supplemental File 4: Appendix D — Section on Swab Sampling from the Lab Journal.

INSTRUCTIONS FOR COLLECTING SAMPLES
Collect the swabs as close to your departure for the practical as possible, but definitely no more than 4 hours before the start of the practical. The shorter the exposure of bacteria and vaginal material to room temperature, the better the results and the greater the chance of isolating *crispatus* bacteria. **If you need more than 4 hours of travel time, you can also collect the samples at the practical location before it begins**.

It's not a problem if you experience bleeding, discharge, or an infection at the time of collection. In the 24 hours leading up to the collection, we ask that you refrain from having unprotected (without a condom) sex, as this can alter the results and make it more challenging to isolate bacteria.

In the box, you will find swabs, two vials of liquid, and a vaginal pH test. Please read the **instructions** below.

You'll want to complete a total of 3 tasks:
- **Collect a swab to gather bacteria** from your vagina and dissolve it in the vial with the pink cap.
- **Collect a swab to gather genetic material** from bacteria in your vagina and dissolve it in the vial with the white cap.
- **Perform a pH** test to measure the acidity of your vagina.

1. We begin with the swab that is with the vial with the pink cap. The vial contains a protective buffer that helps the bacteria stay alive as much as possible, so they can grow when we spread them on a growth medium later.

- First, wash your hands with water. Use a clean surface. Unscrew the cap of the vial and place the cap upside down (so, bottom up) on a clean surface. You can place the vial in the blue rack provided in the collection kit to prevent it from tipping over.

- Take the swab out of its packaging. Important: do not touch the white end with the cotton with your hands. Assume a comfortable position, like when inserting a tampon. You can use the swab while sitting, standing, or lying down.

- Use one hand to spread your labia. Gently insert the end with the cotton a few centimetres into the vagina, until about one-third of the stick is in. This should not cause any pain.

- Rotate the swab and count to 20 seconds. This collects fluid from the vagina, containing bacteria and cells from the mucous membrane. Remove the swab from your vagina afterward. Try not to touch your skin outside the vagina, labia, or thigh with the swab. Do not touch the swab with your hands.

- Place the part with the cotton in the vial and gently rotate it. Press all sides of the swab against the side. Count to 60 seconds to ensure as much fluid from the vagina as possible ends up in the vial. Discard the swab after this.

- Screw the vial shut, still without touching the opening of the vial or the bottom of the cap.

2. Now, we will collect the swab with the vial with the white cap in the same manner.

- The liquid in the vial with the white cap contains a substance that protects the genetic material (DNA). We need undamaged DNA to determine which bacteria are present.

- Collect this swab exactly like the first one. Rotate the swab again for 60 seconds in the liquid to get as much vaginal fluid from the swab into the vial. You can also discard this swab afterward.

3. Now you can perform the vaginal pH test:
- Collect this swab exactly like the earlier swabs.

- Spread it onto the paper provided on the pH card in the box. If there is any fluid on the swab, ensure to transfer as much as possible onto the paper.

- Compare the colour of the fluid on the paper with the colour chart provided.

- Record the acidity on the following page. If you are unsure or if the strip doesn't change colour, for example, because you couldn't get enough fluid from the swab, don't worry! Make a note of it.

- You can optionally affix the paper with a piece of transparent tape.

- Write the date and your pseudonym on the two vials and on this booklet.

With the two vials, a completed questionnaire, and the pH test result noted in this booklet, you are now fully prepared to participate in the practical.

Please record your vaginal pH here.

Please use this space to document any additional experiences or noteworthy observations during the self-collection process. We also welcome your ideas and suggestions for improvement!

Supplemental File 5: Appendix E — Consent form *crispatus* practical

I understand the nature of the research and the purposes of the practical.

I agree to participate in a practical with a maximum of 15 other citizen scientists and up to 5 supervisors/researchers, where these participants may have access to my vaginal samples, view data on vaginal pH, microscopy, and bacteria isolation. Both supervisors and other citizen scientists will assist me in the processing and analysis.

I consent to the collection of special personal data, such as questions about my ethnicity and hormonal status, and the storage of any extracted DNA in my sample, with the understanding that the DNA of the microbes in the vagina will be used for research.

I consent to the processing of this and other data for research purposes by the Vrije Universiteit Amsterdam. These research data will be photographed in my lab journal, stored on a secure laptop, accompanied by the self-chosen pseudonym under which I am known in this study. Questionnaires with special personal data (such as ethnicity), accompanied by a pseudonym, will be collected via the 'Qualtrics' program and the data will be stored on that server for 90 days before being deleted. After the practical, all data will be stored for 10 years in an online secure research environment of the Vrije Universiteit Amsterdam.

I agree to the storage of my contact information by the Vrije Universiteit Amsterdam for the duration of this project (2 years), and I agree that this data may be used to contact me in the future for:

- participation in the hackathon (joint design session),
- a future colonisation study or other follow-up studies,
- the final conference where the project's results will be shared.

I agree to the use of my pseudonymised non-identifiable research data and samples for future research purposes within the Vrije Universiteit Amsterdam.

I understand that there is no compensation for participating in this research.

I agree to the processing of data on bacterial isolation in the license agreement for notification and invitation to participate in the donor panel of Stichting *crispatus*, which will provide advice to the Foundation for the application of the strain collection in the future.

I agree to microbiome analysis of a vaginal swab by partner Baseclear and the sharing of this information with the Vrije Universiteit Amsterdam for research purposes. Baseclear will not receive answers to the questionnaire or identifiable personal data but will receive your pseudonym.

I understand that the analyses conducted in the practical are not medical standards but experimental research methods, that the researchers and supervisors are not medically trained, and that the research results cannot be used to make medical decisions.

I understand that I can always withdraw from participation in the practical, whether I have received the collection kit, taken the samples, arrived at the practical location, or after the practical is completed. I understand that my participation does not obligate me to participate in future studies.

I understand that once I have isolated the *crispatus* bacteria and signed the license agreement, I can no longer withdraw these bacteria. I can still choose to discontinue my participation in the donor panel at any time, and I will still be entitled to my share of the proceeds from sublicense agreements.

Given the above, I agree to participate in this research.

NAME SIGNATURE
DATE

PLACE

Supplemental File 6: Appendix F — Section on Microscopy from the Lab Journal.

Day I Instruction: Microscopy

Materials: Glass slides, Coverslips, Pen

Using a Pasteur pipette, place a single drop from the vial with the pink cap in the centre of the glass slide. Put on safety goggles. Pass the glass slide through a flame with tongs three times until the drop has dried. Add a small drop of red dye (safranin) to the dry drop. Place a smaller piece of glass, known as a 'coverslip,' over it. The assistant will guide you in making the bacteria visible using a 1000x magnification with the help of an oil drop (1000x). Dispose of the Pasteur pipette in the waste bin on the table.

Describe what you see under the microscope. Can you recognise the bacteria? Do you recognise any other cells? What is the shape of the bacteria? Are there many or few? Feel free to attach a print if available.

Supplemental File 7: Appendix G — Section on Identification and Isolation from the Lab Journal.

Day II Instruction: Finding and isolating *crispatus*

Recognising *crispatus* colonies
First, we will examine the colonies that have formed on the growth media. We identify *crispatus* colonies based on their shape. For this, we use sample plates of *crispatus* colonies and plates with other lactobacilli (*plantarum*, *gasseri*, *jensenii*, *bulgaricus*).

- Place the closed plate in front of you with the lid facing down next to the comparison plates (with different lactic acid bacteria).

- Select a number of colonies (up to 8) that you find most similar to the comparison material on the *crispatus* plate. Do not select the very smallest colonies, but preferably larger ones that are as separate as possible, consisting of one round.

- Draw circles around the colonies with a marker and label them (1-8). We will now examine and subculture these colonies.

- Describe on the next page how your colonies look. Describe the amount, shape, diversity, colour, and size.

LAMP Test
Now we will perform a special LAMP test to see if the colonies are indeed *crispatus*. In this experiment, we copy DNA using unique DNA primers. The primers can only attach to the DNA of *crispatus*, so only if the colony is a *crispatus* bacterium will the DNA be copied. If the colour in the tube changes from red to yellow, the test is positive. This indicates that the DNA has been copied in the tube containing those bacteria, confirming the presence of grown crispatus bacteria.

- You will receive a maximum of 8 larger tubes (vials) with water and a maximum of 8 small tubes (PCR tubes) with red dye. The 'copying device' with the primers has been added to these. We will also use a water bath at 65 degrees with floats.

- Write the numbers corresponding to the colonies you want to examine on the small tubes with red liquid and on the larger tubes (vials) with water.

Please be aware that some colonies on the growth medium may consist of dangerous bacteria. Therefore, make sure to wear a glove on the hand you use to touch the plate, and do not touch anything else with it. On the other hand, do not wear a glove. Do not touch your face or clothing during this experiment.

To ensure that the lactic acid from the lactic acid bacteria does not interfere with the experiment, we will first wash the bacteria from the colonies with water.

- Remove the stickers on the petri dish and place it in front of you with your gloved hand. Lift the lid off. Using your other hand (ungloved), pick up a white plastic loop and touch only the colony with it.

- Now transfer the material into the larger tube (vial) with water and rotate the stick between your thumb and forefinger for about 10 seconds.

- With the same loop, transfer a tiny droplet of washed bacteria into a tube with red liquid.

You should only touch the tubes with your gloved hand, and handle the loops with the hand without a glove. The assistant will help you with this.

- Once you have transferred all the colonies you want to examine via the vials (tubes with water) into the red liquid, now close the small tubes with red liquid and place them in the floats in the water bath. Are the numbers still clearly visible? If not, write them down again clearly.

- After three quarters of an hour, check the colour in the tubes in the floats. If the liquid has turned yellow, it likely means the colony is a *crispatus* colony. Record this information in your lab journal on the next page. The colour of the tubes will also be photographed. Do not open the tubes, but dispose of them sealed in the waste bins on the lab table.

Please record your overall impression of the growth medium. Do you observe numerous colonies or only a few? Do the colonies resemble each other? Are there variations in terms of shape, size, colour, or texture? Do they resemble any of the example plates?

Please record here the colonies you are further analysing. Assign each colony a number and note its shape, size, colour, and texture. Also, indicate whether the colony turns red or yellow in the lamp test.

Supplemental File 8: Appendix H — Loop-mediated isothermal amplification protocol

Lactobacillus crispatus colony identification with colorimetric Loop mediated isothermal amplification (LAMP) protocol

January 2023
Shardelice Illidge, Remco Kort, Rosanne Hertzberger
A-life, Vrije Universiteit Amsterdam

Here we present a protocol and validation for a test that allows the rapid identification of *Lactobacillus crispatus* colonies grown from vaginal samples. In our project we aim to use this method in a citizen science context (see more information on crispatus.org). This method could be adjusted for the identification of other microbes and could also be used in low-resource settings.

Introduction

The Loop-mediated isothermal amplification method (LAMP) is a DNA amplification method with high specificity, amplification efficiency and allows a reaction to take place at a constant temperature, 65 ˚C. It uses 4 primers and a DNA polymerase with strand displacement activity. The 4 primers recognise 6 different regions on the target DNA allowing for a rapid amplification process [1]. To accelerate the reaction one or two additional primers, called Loop Forward (LF) and Loop Backward (LB), are added to the mix. The WarmStart® Colorimetric Master Mix (New England Biolabs, Ipswich, Massachusetts, United States) that is used in this protocol, has a low TRIS buffer concentration (pH 8) and contains a pH indicator (phenol red) that changes colour from red to yellow below a pH of 6,8. This colour change is attributed to the release of protons during the amplification process, causing a decrease in pH. The amplified product can be easily identified because of this colour change [2,3].

A previous study designed specific LAMP primers for *Lactobacillus crispatus,* demonstrating the LAMP test's capability to detect as little as 10 pg of *Lactobacillus crispatus* template DNA [2]. To mitigate the risk of false positive results arising from lactic acid-producing bacteria, a precautionary dilution step was implemented. This involved the addition of 100 µL of Milli-Q water to reduce the interference of lactic acid associated with the colony, while still being able to perform the assay with a much lower concentration of DNA.

Materials

- WarmStart® Colorimetric LAMP 2x Master Mix (DNA&RNA)
- *L. crispatus* LAMP primers [2]**,** prepare a 1 mM working stock
  - FIP CGGTTTGCGGTACGGGTATGTCGTGTGGTAATCACACTGCCA
  - BIP AGGAACTCGGCAAAATGACCCCGGCTAACCAATCTCTTGGCT
  - F3 ACGAGTTGTGAAGAGGAGTGA
  - B3 TGTTTGGGCCTATTCACTGC
  - LB TAACTTCGGAAGAAGGGGTGCT
- MilliQ
- Water bath
- Plastic toothpick
- PCR tubes

Procedure

1. Set the temperature of the water bath to 65˚C.

Determine how many samples you will be analysing and the number of the PCR tubes you might need, including the positive and negative controls. Use a known *crispatus* containing material (culture, strain or vaginal sample) as a positive control. Use water as a negative control.

Prepare your master mix in PCR tubes as follows:

	Volume per reaction
Warmstart	12,5 µl
Primer mix*	2,5 µl
Milli-Q	10 µl
Total	**25 µl**

Warning: The LAMP test produces large amounts of DNA. Even when working in a very clean manner, it is important to prepare the master mix in a separate room, to prevent positive results.

2. Gently scoop up a single well-isolated colony with the plastic loop or tip and suspend it in a 1,5 ml tube containing 100 micro litres of water. Spin the loop between thumb and index finger to dislodge the bacteria. The water will become turbid.

3. Add a small amount of this bacterial suspension, by dipping a second plastic toothpick in the solution and transfer to the LAMP master mix. Mix the solution with the toothpick. If there are drops at the top of your tube, gently slide them down with your toothpick.

4. Incubate the PCR tubes in the water bath for 30 minutes at 65˚C. If after 30 minutes there are samples that are orange, let them incubate for another 10 minutes.

Result analysis

5. The samples can be assessed by colour. The negative samples should remain red. The starting mix will turn from red to bright yellow if it is positive for *L. crispatus.* An orange result should be interpreted as negative.

When this method is used for *Lactobacillus crispatus* isolation, continue by restreaking the remaining colony on a new MRS (de Man, Rogosa and Sharpe) agarose plate and incubate anaerobically for a minimum of 48 hours. The LAMP protocol could be repeated to reaffirm the previous results. Colonies from this secondary plate are then inoculated into MRS liquid medium. After incubating the culture for 24-48 hours (anaerobically), we prepared a glycerol stock in a cryovial. We used a 60% (v/v) glycerol stock solution by pipetting 1 ml of liquid culture and 500 µl of 60% glycerol in a cryotube vials (the final glycerol concentration is 20%), flash freeze them in liquid nitrogen and store them at -80˚C.

*Prepare your primer mix prior to the experiment as follows:

Primer	**Volume (µL)**
Fip	8
Bip	8
LB	4
F3	1
B3	1

Primer	Volume (µL)
MilliQ	478
Total volume	500

Verification of colorimetric LAMP-test results

We grew two previously isolated and sequenced *L. crispatus* strains RL09 and RL10 [4] on tryptic soy broth (TSB) agar. We also streaked vaginal swabs with different Nugent scores on Tryptic Soy Broth agarose plates supplemented with 10% horse serum, 1g/L tween 80 and 10g/L glucose and pH set to 5 with 10% acetic acid. The inoculated agarose plates were incubated anaerobically at 37˚C for 48-72 hours. We selected the colonies based on their morphology and confirmed identity with the LAMP test. Colony PCR with specific *L. crispatus* primers was used to validate the LAMP test results. The primers targeting the 16S rRNA that were used in this assay were derived from Kusters et al. [5] and produced an amplicon of 145 bp.

Forward: AACTAACAGATTTACTTCGGTAATGA
Reverse: AGCTGATCATGCGATCTGC

The identity of the positive and negative colonies were further confirmed by MALDI-TOF biotyping at the Streeklaboratorium Amsterdam.

We tested LAMP on other *Lactobacillus* species such as *L. plantarum, L. delbrueckii* subsp. *bulgaricus* and *L. gasseri* cultures as a control. All of these species tested negative in colony PCR assays as well. We also tested 134 colonies during several citizen science practicals, and compared the outcome to the colony PCR using *L. crispatus*-specific primers (Kusters et al.).The band was observed in 61 colonies, which correlated with the outcome of the LAMP test with 100% accuracy (see Figure 1 for a subset of these colony PCR results).

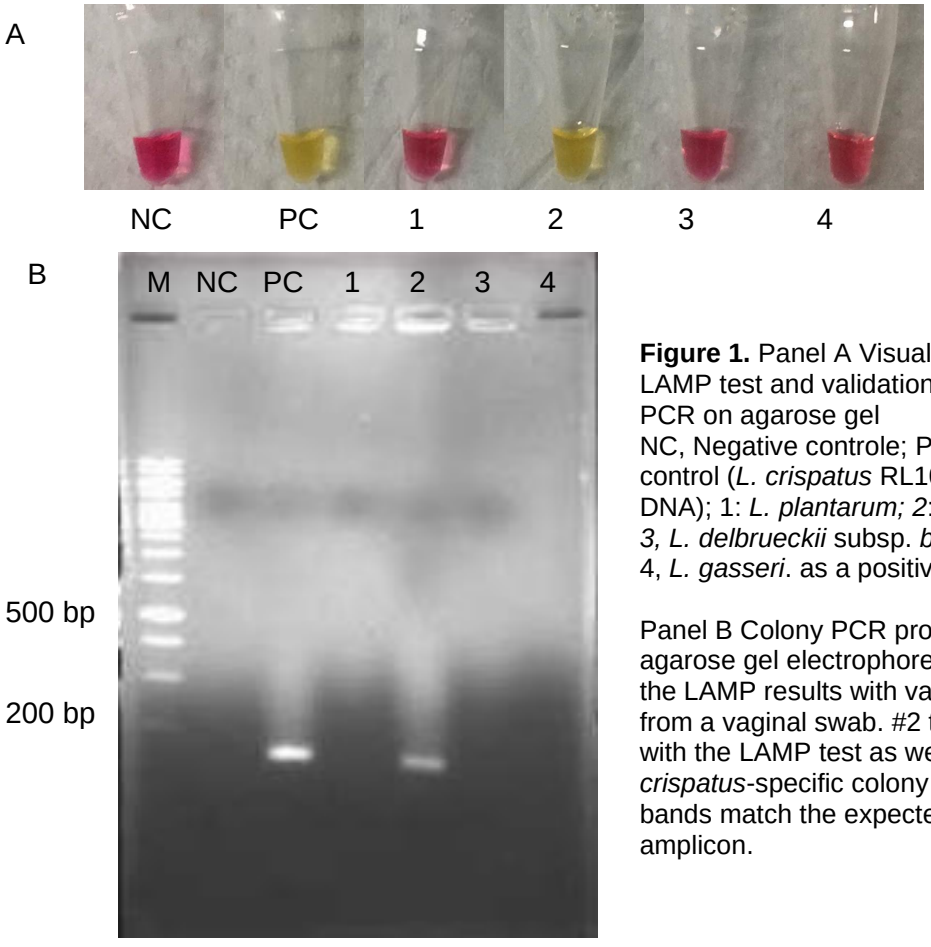

Figure 1. Panel A Visualisation of LAMP test and validation with colony PCR on agarose gel
NC, Negative controle; PC, positive control (*L. crispatus* RL10 genomic DNA); 1: *L. plantarum; 2*: *L. crispatus; 3, L. delbrueckii* subsp. *bulgaricus* and 4, *L. gasseri*. as a positive control; .

Panel B Colony PCR products on agarose gel electrophoresis to validate the LAMP results with various colonies from a vaginal swab. #2 tested positive with the LAMP test as well as with the *crispatus*-specific colony PCR. The bands match the expected 145 amplicon.

Comments

There are different ways a false positive or negative can be obtained. The assay is sensitive to acidic solutions or templates due to the pH indicator in the mix. It is important to eliminate all traces of acidity prior to the assay. Lactic acid-producing organisms should undergo dilution in water to reduce the concentration of lactic acid associated with the colony, as described in step 2. For a reliable test, make sure to use a non-absorbent material for the inoculation of the master mix. A wooden toothpick would absorb the water content, acid included, and turn the LAMP master mix yellow before incubating your samples, resulting in a false positive result. The samples should not be incubated for longer than 45 minutes. A longer incubation period may lead to false positive results. Consider the size of the colony and the amount of water needed to dilute the lactic acid. For smaller colonies the amount of water can be lowered. We have noticed that the use of isolated DNA in Tris-HCl buffer can turn a positive result orange instead of yellow. This may be due to the buffer's neutralising effect.

Citizen science / low resource adjustments

A few replacements that could be introduced to adjust this protocol to low-resource setting:

- Instead of a water bath, kitchen equipment could be used to create the constant temperature such as a 'sous-vide.'
- Vittorio Saggiomo has also introduced recycled Nespresso cups filled with phase-change material (T-cup) and a 3d printed tube holder. The phase change material is melted in boiling water, and will stay at the desired temperature for hours.

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Supplemental File 9: Appendix I — Section on Sample Plating from the Lab Journal.

Day I Instruction: Isolating and Cultivating Bacteria

Instruction: Spreading the Contents of the Vial with the Pink Cap on a Growth Medium

A portion of the vial with the pink cap is spread onto a growth medium and placed in an airtight (anaerobic) container in an incubator at 37 degrees Celsius.

The assistant will provide you with a growth medium in a transparent dish, also known as a petri dish. During this practical, use one hand to handle both the vial and the petri dish. Wear a **glove** on this hand.

- Place the petri dish with the lid facing up on the table. Open the lid and position it with the opening facing upward. Mix the contents of the pink vial by turning it upside down a few times.

- Set the vial down and place the cap with the opening facing up. **Try not to touch** the opening of the two lids, the vial, or the petri dish.

- Now, with your glove-free hand, take a plastic sterile loop from the packaging. Dip it into the vial and then spread it over the growth medium. Ensure that as much of the plastic loop comes into contact with as much surface area of the growth medium as possible.

Note: Try to touch the growth medium as lightly as possible with the sterile loop and avoid piercing it as much as possible. This may not be very easy, and if the growth medium isn't entirely intact, it's not a problem.

- Again, move the loop from left to right and from top to bottom over the growth medium to spread the bacteria as much as possible. Even if you can no longer see vaginal material with the naked eye, bacteria can still end up in those spots and grow. Dispose of the loop in the waste bin on the table.

- Turn the petri dish upside down onto the lid and secure it with two labels. Write the **date** and your chosen **pseudonym** with a pen on the label.

- Now, place the petri dish upside down (with the growth medium facing upward) on the rack. Later, we will place all the growth mediums in an oxygen-free container, where they will be cultivated with added CO2. These are the ideal conditions for *crispatus* bacteria.

We will repeat this process with a bit more liquid on a second plate:

- Use a plastic Pasteur pipette with your glove-free hand. Draw up a small amount of liquid from the pink vial and release about three drops onto the plate. Now, take a blue plastic 'hockey stick' with your glove-free hand and gently spread the liquid to all corners. Try to keep the base intact. Continue spreading until most of the liquid is absorbed into the growth medium. Dispose of the hockey stick in the waste bin on the table.

- Turn the glove inside out on your hand by pulling it up from the edge with your bare hand and discard it. Minimise touching the working area of the glove. Wash your hands thoroughly with soap afterwards.

Day II Instruction: Transferring *crispatus* Bacteria to a New Growth Medium

Now we are going to transfer the *crispatus* colonies to a new growth medium so that they can be cultured and stored for further research.

- The assistant will provide you with a new growth medium. Use a marker to draw squares on this new growth medium, squares where you will streak each colony. Note down the corresponding number of the colony you want to streak.

- Open both growth media with your gloved hand and locate the colony that turned yellow in the LAMP test (indicating it is very likely to be *crispatus*).

- With a new loop, touch this colony and streak it onto the new growth medium, in the square where you have noted down the corresponding number. Dispose of the loop.

- Seal this growth medium and hand it to the assistant. Clean the surface of the table with 70% alcohol and dispose of everything in the waste bins on the lab table.

- This new growth medium will be placed in an anaerobic jar (without oxygen) and incubated at 37 degrees Celsius.

If everything has gone well, your isolated *crispatus* bacteria are now growing on this growth medium. At the Vrije Universiteit Amsterdam, we will continue to cultivate them in liquid broth and conduct research based on the hereditary material (DNA) of these bacteria.

In the future, we may also use the bacteria to investigate whether they can help other women.

Thank you for your participation!