BiTTE®-iE instruction manual

May 2024 edition

1. Precautions for use (for slides and images to be estimated)

(1)Summary

Capture the microscopic field of view of a Gram-stained slides at 1,000x magnification as an image through a smartphone camera for AI-based estimation.
*When using BiTTE-iE's camera function, images can be captured with a digital zoom of 1.9x.

You can also use images saved on your smartphone.
*Please check the recommended staining method and recommended images.

・Recommended staining method:Bartholomew ＆ Mittwer methods
・Recommended images:
・Input image size: W3024 x H4032 pixels
・Equivalent to 12 million pixels or more
・Image captured at 1.9x digital zoom

(2)Details

We recommend the use of microscope slides and images created according to the following procedure.

- Recommended microscope slides:
  - MATSUNAMI GLASS IND., LTD.
    - Basic Frost White Water Rim Polished 7Ft1.0
    - code:S2541-7F
  - MUTO PURE CHEMICALS CO.,LTD.
    - Star frost slide glass
    - code:511611

- Recommended staining method:
  - Bartholomew ＆ Mittwer method
    - staining reagent
      - MUTO PURE CHEMICALS CO.,LTD.

        - Bartholomew & Mittwer M staining kit (especially recommended)
    - FUJIFILM Wako Pure Chemical Corporation
        - Gram staining solution neo-B&M Wako

- staining procedures
    - Creating Slides
        - Collect the necessary amount for the object to be observed.
        - Prepare microscope slides for observation.

    - staining procedures
        - **Methanol is dropped onto a microscope slide**
          Bacteria are fixed to microscope slides by dehydration and protein coagulation.
            - Wait 1 minute.
            - Allow to dry completely, eliminating excess ethanol.
        - **Drop crystal violet on a microscope slide**
            - Wait 30 seconds to 1 minute.
            - Rinse with water.
        - **Drip iodine**
            - Wait 30 seconds to 1 minute.
            - Rinse with water.
        - **Gently drop in decolorizing solution**
            - Wait a few seconds to a few dozen seconds (shake the glass slide to eliminate uneven staining).
            - Rinse with water.
        - **Drip fuchsin**
            - Wait 30 seconds to 1 minute.
            - Rinse with water.
            - Allow to dry.

- Observation
    - Recommended microscope
        - Optical microscope with adjustable optical axis
            - Olympus Corporation
                - BX-51
            - NIKON CORPORATION
                - ECLIPSE Ci：Nikon
                - ECLIPSE Si：Nikon
        - Recommended objective lens
            - "non-cover type" lenses for 100x lenses are rcommend

        - Cover-type lenses have a blurred focus due to the correction of the refractive index of the cover glass.

        - MPLAPON 100XO2：OLIMPUS
        - CFI Plan Apo NCG 100xh Oil：Nikon
    - Recommended immersion oil
        - Cat. No.15671；Immersion oil：MUTO PURE CHEMICALS CO.,LTD.
- Initial setup of microscope
    - Viewing aperture and condenser position adjustment (resolution adjustment)
        - Narrow the field of view aperture to produce a round shadow.
        - Adjust the height of the condenser so that the outline of the shadow is polygonal.
    - Aperture Adjustment (Adjustment of the degree of light collection)
        - Adjust the aperture diaphragm value to the value obtained by multiplying the numerical aperture of the objective lens by 0.7 to 0.8.
        - The value of the aperture diaphragm should be 0.6 or higher.
    - Eyepiece adjustment (diopter correction)
        - Vision Correction
            - Adjust the memory of both eyepieces to 0.
            - Adjust the stage to focus on the sample.
            - Look through one eyepiece at a time, adjust the blurred eyepiece and bring the sample into focus.
        - Set the same focal distance.
            - Remove one eyepiece and focus on the sample with the remaining eyepiece and ×40 objective lens.
            - i: Attach both eyepieces and bring the sample into focus again with the ×10 objective lens.
            - ii: Bring the sample into focus again with the ×40 objective lens.
        - Repeat i,ii to bring the sample into focus even if the magnification is changed.

- How to use microscope
    - Place the stained slides on the microscope.
    - Lower the stage with slides to the lowest position with the coarse adjustment screw.
    - Adjust the objective lens to the lowest magnification (x4 objective lens)
    - Gradually raise the stage using the coarse adjustment screw to bring the microscope into focus with the specimen.
    - Change the magnification of the objective lens to 10x.

- Adjust the stage with the fine adjustment screw to bring the sample into focus.
- Magnification of the objective lens is changed to 40x.
- Adjust the stage with the fine adjustment screw to bring the sample into focus.
- Dab a small amount of oil-soaked oil on the sample and replace the objective lens with the 100x objective lens.
- Adjust the fine adjustment screw to focus on the sample and observe the sample.

- Imaging Model and Method (Smartphone)
  - iPhone that meets the following requirements
  - Capable of camera shooting with input image size of W3024 x H4032
    - (equivalent to 12 million or more camera pixels)
  - Models capable of capturing images with a digital zoom of 1.9x
    - Models with dual and triple camera systems
  - Jpeg image format recommended

  - adapter
    - NexyZ universal smartphone adapter (CELESTRON, CA, USA), to an optical microscope to focus the image from the microscope eyepiece.

  - Using the camera function of BiTTE-iE, take images with the bacteria to be determined as close to the center of the screen as possible.
    - When using the camera function of BiTTE-iE, the image can be taken with a digital zoom of 1.9x.

- Imaging Environment
  - Places where there is no natural light
  - Other points to note when taking images

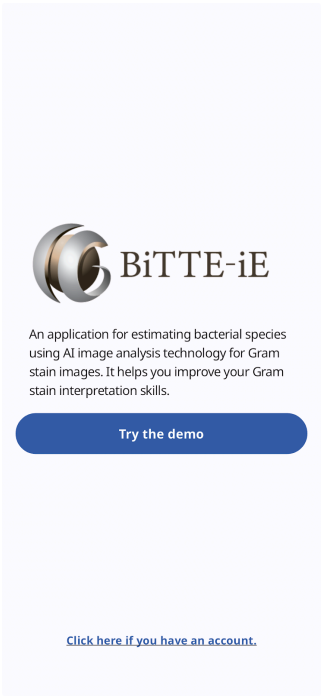
- When imaging with a Nikon microscope, the image may have a light green or yellowish tint.
- Restarting the camera application on the smartphone will return the color to transparent.

- (3)Other
  - In the case of an estimation target other than microorganisms observed with an optical microscope, or in the case of use of this product based on an inappropriate method that does not comply with the contents of this instruction manual, there is a possibility that the product will not output the estimation results it is aiming for.
    - Example)
      - Images taken by other than optical microscopes
      - General images not related to microbiological inspection
      - Images taken with a smartphone camera while the field of view observed with an optical microscope is displayed on a PC monitor
      - Glass slides made using methods other than those recommended

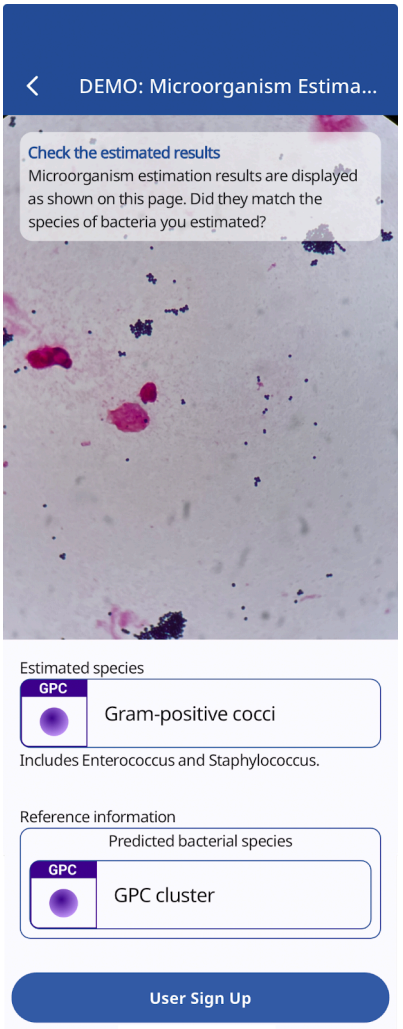
2. How to operate the app

● 1) Please download the app from the QR code.	iOS Android

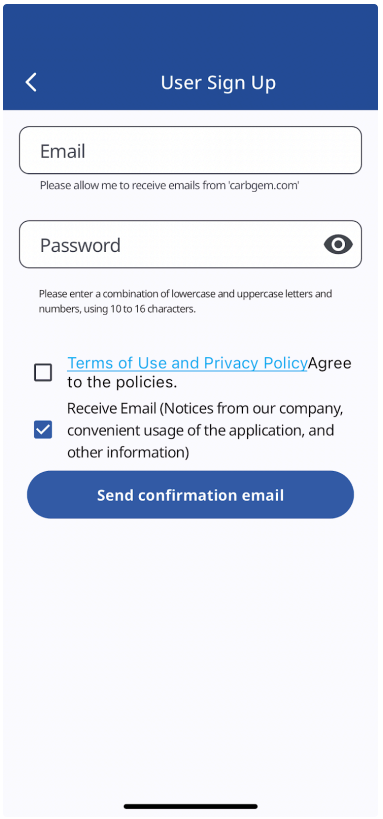
- 2)
  After installation is complete, launch the application and tap "Try the Demo”.

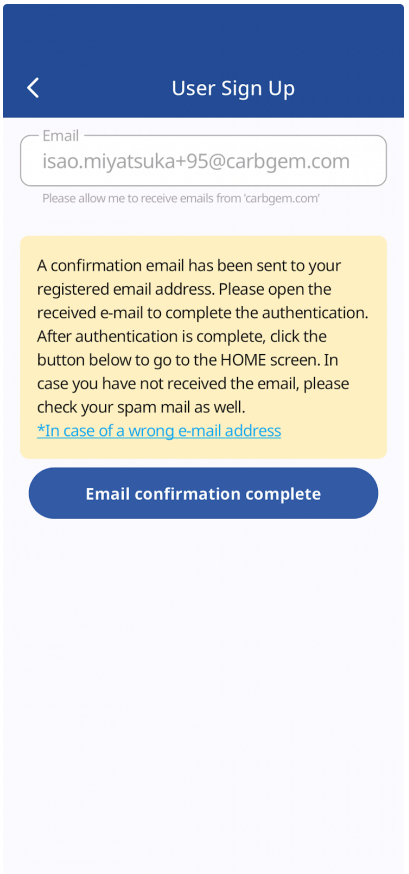


- 3)AI Estimation Demo
  - You can check the AI Estimation Demo. Select an image and press the "Start" button.
  - Confirm the estimation result. Click the "User Sign Up" button.
    - By registering as a user, you can estimate microorganisms in the microscopic field of view or your own images.



- 4)User Registration
  - Enter the e-mail address you wish to use.
  - Set a password to log in.
  - Agree to the Terms of Use and Privacy Policy, then tap the "Send confirmation email" button.





- 5)　You will receive an email with a link to authenticate your email to your registered email address, click on the link you received.

（Content of email sent）

Sender：bitte@carbgem.com
Subject：Verify your email for BiTTE®-iE (CarbGeM Inc.)

Hello,

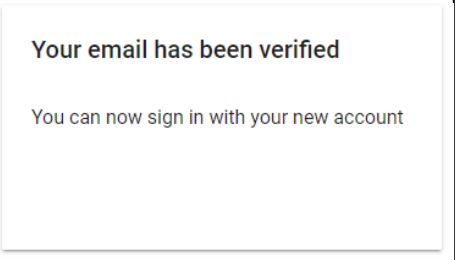
Follow this link to verify your email address.

https://_/auth/action?mode=verifyEmail&oobCode=XX&apiKey=YY&lang=en

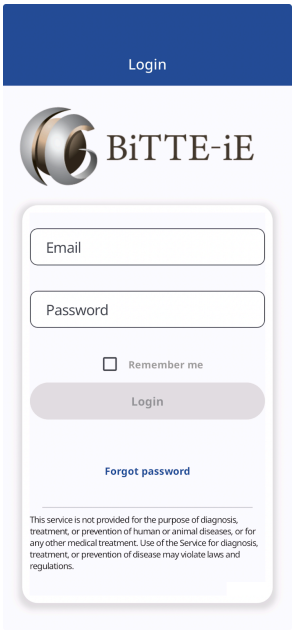
If you didn’t ask to verify this address, you can ignore this email.

Thanks,

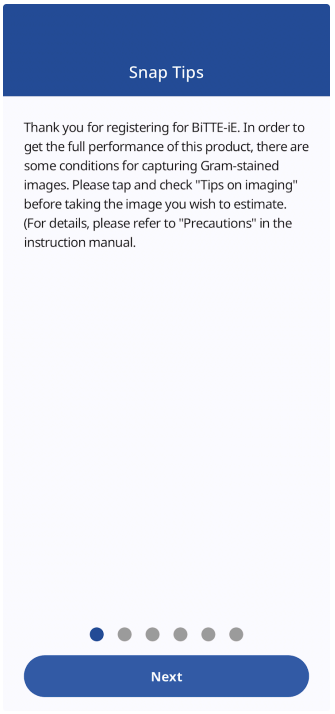
Your BiTTE®-iE (CarbGeM Inc.) team

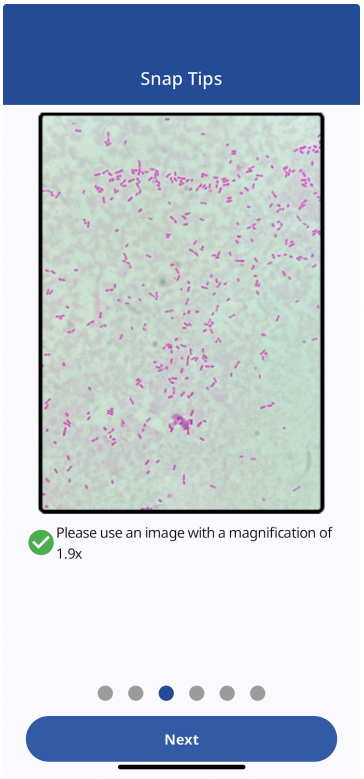


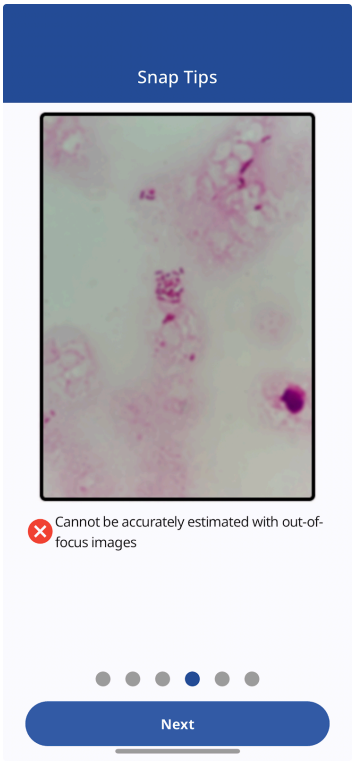
- 6)　Log in with your registered email address and password.

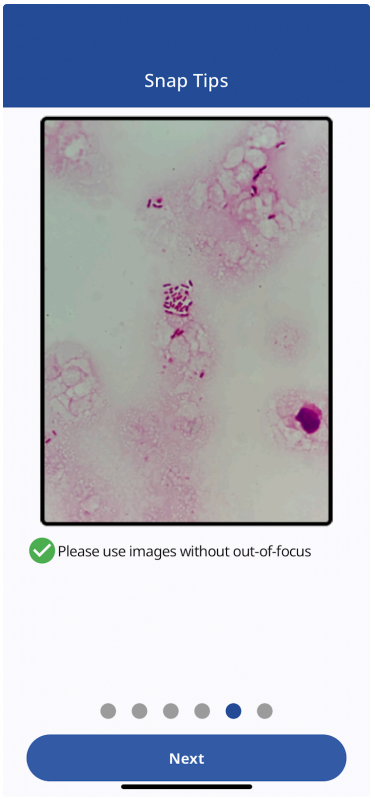


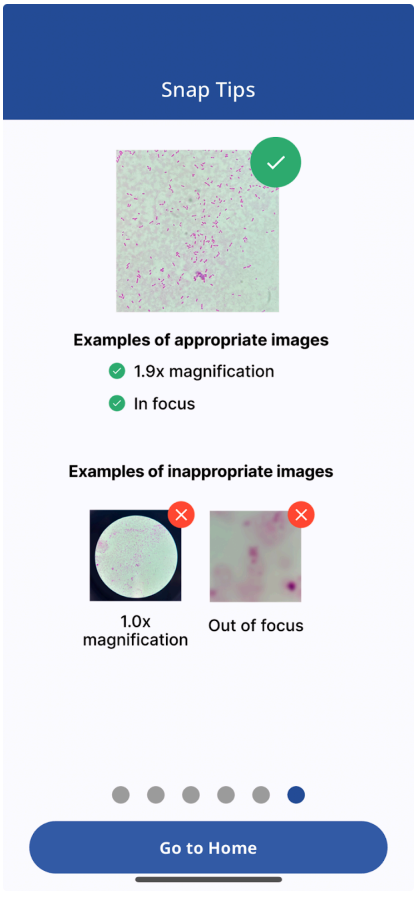
- 7) Instructions on how to use this app will be displayed. Tap the screen and proceed to "Next". (Total of 6 steps)

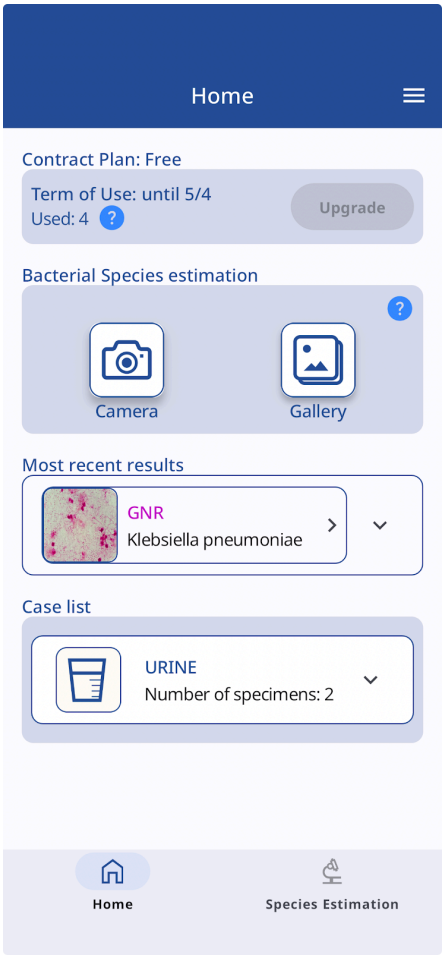
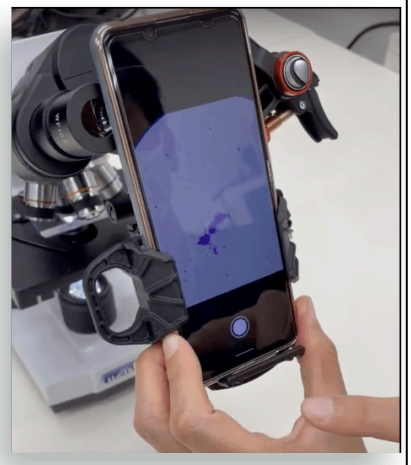



Snap Tips
Please use an image with a magnification of 1.9x
Next


Snap Tips
Cannot be accurately estimated with out-of-focus images
Next

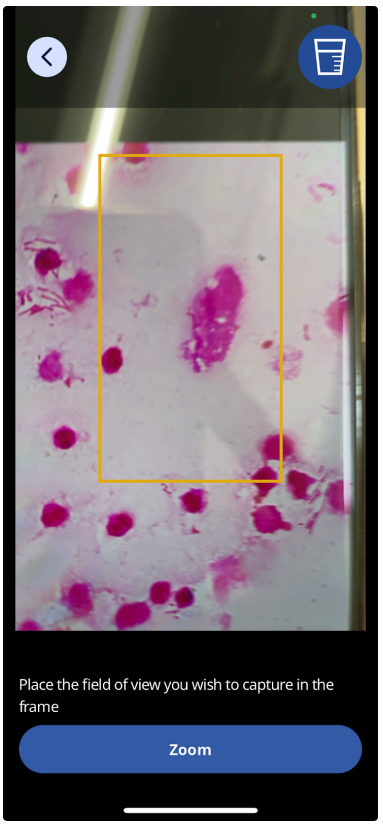

Snap Tips
Please use images without out-of-focus
Next

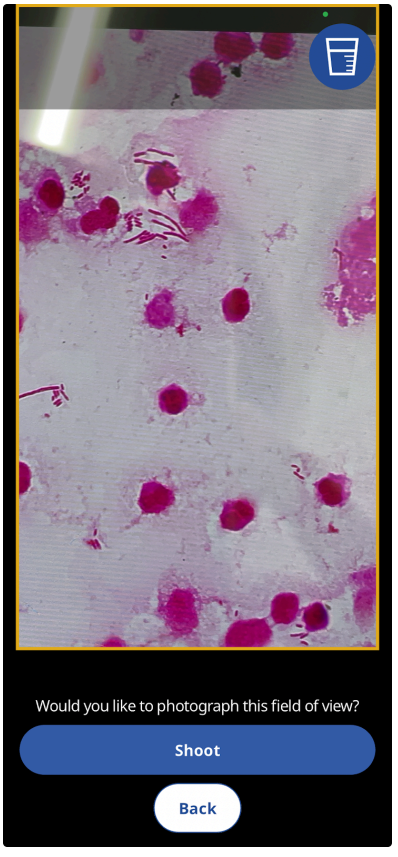

Snap Tips
Examples of appropriate images
1.9x magnification
In focus
Examples of inappropriate images
1.0x magnification
Out of focus
Go to Home

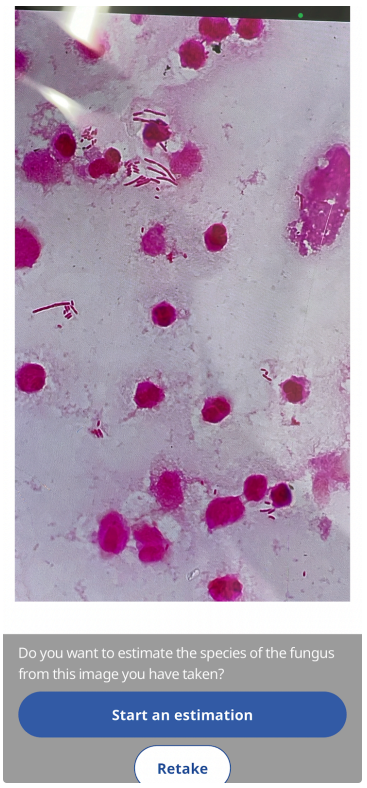
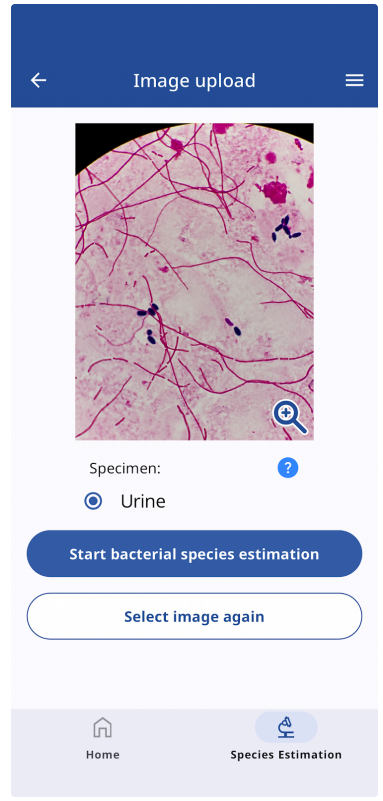
• 8) Access the home screen. Select "Camera" to capture a Gram stained image of the bacteria you wish to estimate the species of.	
• 9) The observation field of view is set to be captured by the camera of a smartphone with this application installed via a mounting device (adapter) to the objective lens of an optical microscope, and take a picture. • Image format is JPEG (compatibility priority) • For a mounting device, NexyZ universal smartphone adapter is used. • For details, please refer to "1. Precautions for Use". a)About when you tap "Camera" on the home screen	

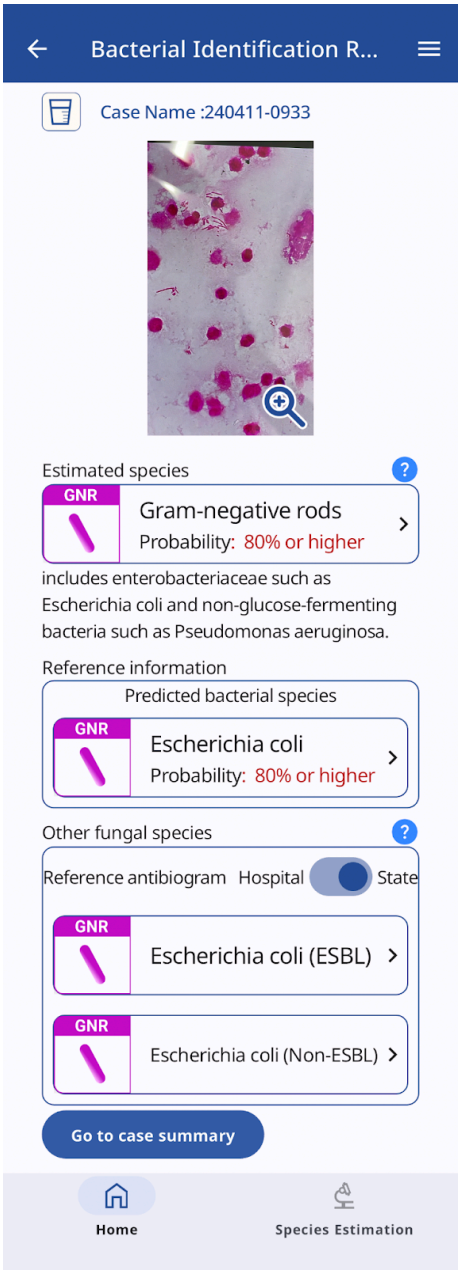
- Adjust the area to be estimated to fit within the orange frame and tap "Zoom".
- Tap the "Shoot" button to take a picture of the field of view you want to estimate.
- Check the shooting screen and press the "Estimate the fungus species" button. Click "Restart" button to start over from the selection of the area.
-
- When using the camera function of BiTTE-iE, the image can be captured with a digital zoom of 1.9x.

- The AI model used can be a model that has been trained on a urine sample.

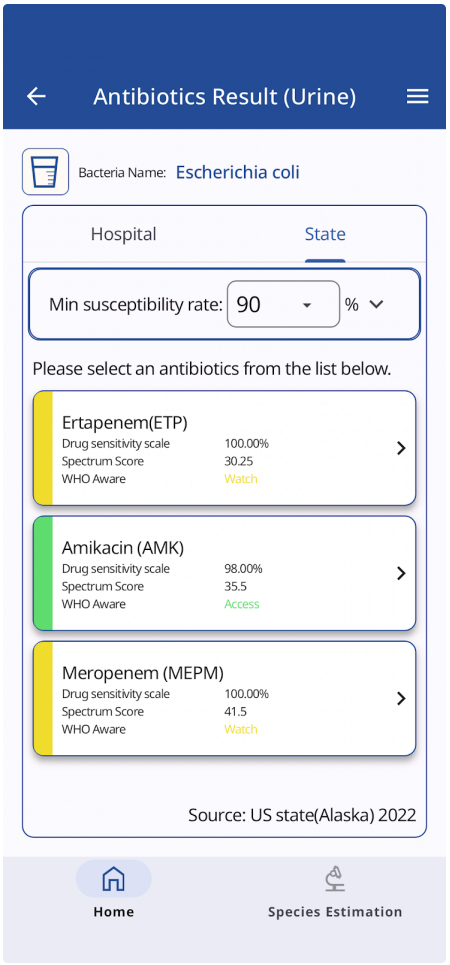




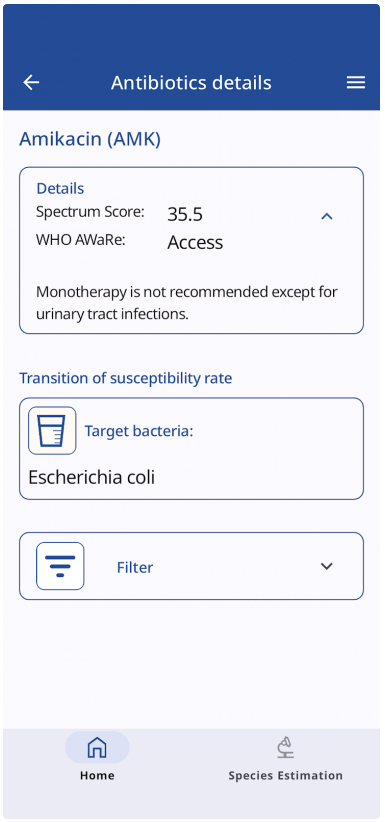
<table>
<tr>
<td></td>
<td> 
</td>
</tr>
<tr>
<td>b)About tapping "Photo" on the home screen
● Select the image you wish to determine the bacterial species from your smartphone, and press "Start Bacteria Species Estimation".
●
● Please refer to the recommended staining method and recommended images for the target images.
○ Recommended staining method:
■ Bartholomew & Mittwer methods
○ Recommended image:
■ Input image size: W3024 x H4032 pixels
● Equivalent to 12 million image pixels or more
■ Images captured at 1.9x digital zoom</td>
<td> 
</td>
</tr>
</table>

• AI model to be used: A model trained on urine specimens is available. •	
• 10) In about 10 seconds, the results of the bacterial species estimation for Classification 1 and Classification 2 are displayed. • In the example on the right, the result of the estimation for Classification 1 is determined to be "Gram-Negative Rod" and the result of Classification 2 is determined to be "Escherichia coli". • The estimation is counted as one use per sample. If an estimation is determined to be "no bacteria" or "multiple bacteria," it will not be counted as one use.	

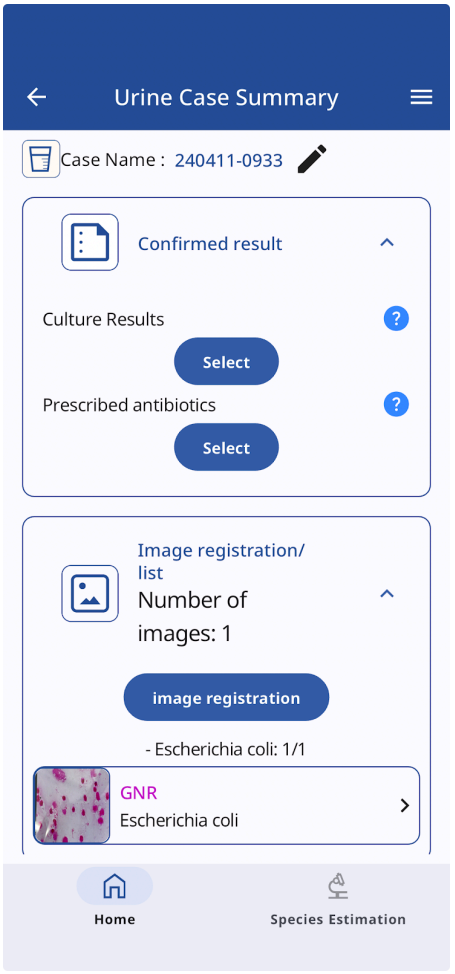
- 11)　A list of candidate antibiotics for the bacterial species is displayed based on the statistical data of antimicrobial susceptibility tests.
- Tap "Escherichia coli" in the example shown in 10) to display the screen as shown on the right.
- By switching tabs, you can view the list of candidate antimicrobial agents based on JANIS statistical data and the list of candidate antibiotics based on the antibiogram unique to the medical institution.
- The antibiograms unique to medical institutions must be imported in advance.
- The list of candidate antibiotics can be sorted by minimum susceptibility rate, spectrum score, and WHO AWaRe classification.
- The list of candidate antibiotics is counted as one use per sample. If the message "No statistical data for antimicrobial susceptibility test" is displayed, it is not counted as one use.
-

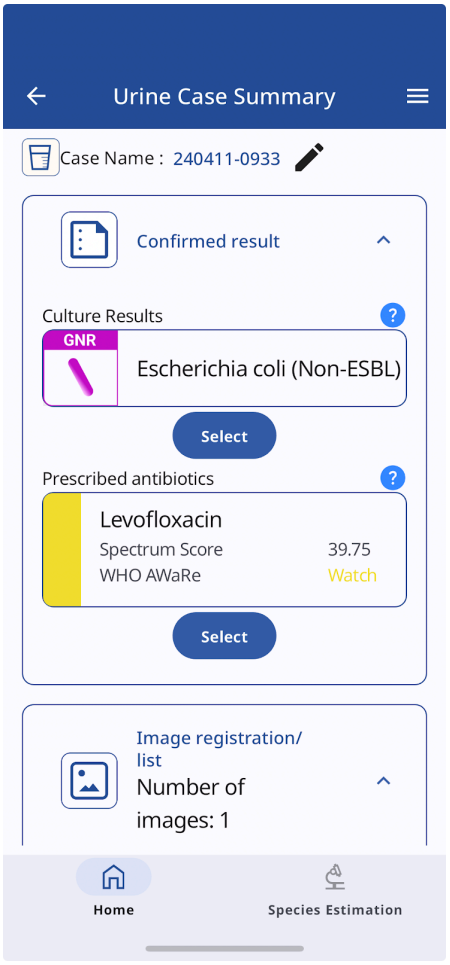


- 12)　By tapping on an antibiotics displayed in the list of candidates, you can confirm detailed information about the antibiotics.

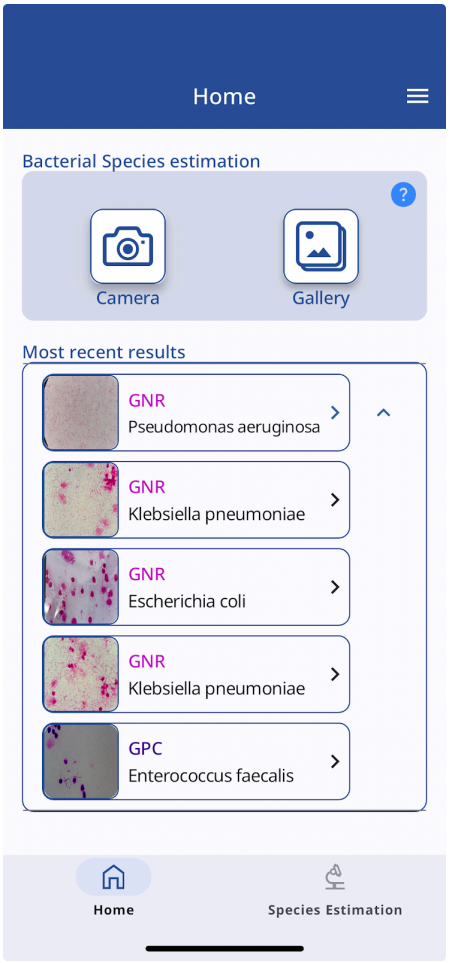


- 13) By clicking "Case Summary" on the screen shown in 10), the screen shown on the right can be displayed.
- By clicking "Image registration", you can save the results of the bacterial species estimation for multiple views of the same sample.
- After the culture test, you can also provide feedback on the bacterial species name of this sample.
- Select the species name by pressing "Select" in the "Culture Results" section of the "Confirmation result" area.
- The feedbacked information will be used to improve the accuracy of the test.
- You can also feed back the information of antimicrobial agents prescribed as Definitive Therapy.
- In the "Prescribed antibiotics" section of the "Confirmation result" area, press "Select" to choose the name of the antibiotics.
-
-

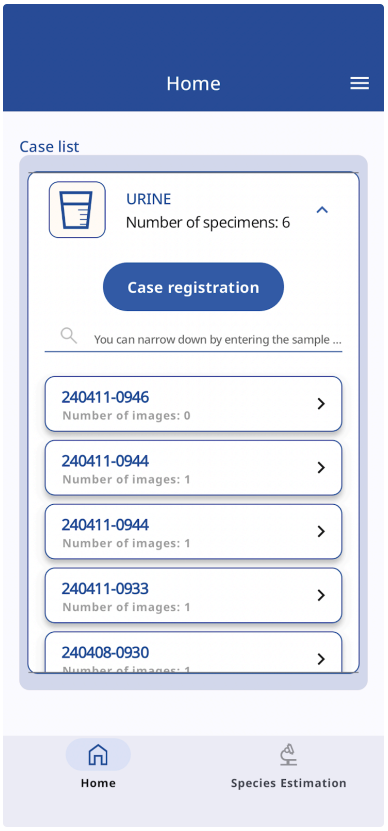



Urine Case Summary
Case Name : 240411-0933
Confirmed result
Culture Results
GNR
Escherichia coli (Non-ESBL)
Select
Prescribed antibiotics
Levofloxacin
Spectrum Score 39.75
WHO AWaRe Watch
Select
Image registration/ list
Number of images: 1
Home
Species Estimation

- 14) Up to 5 most recent results can be displayed on the home screen.
- By clicking on the name of the bacteria, the results can be displayed again.
-



- 15)　In the “Case list” section of the Home screen, you can search and refine samples by entering part of the sample name in the search field.
- Click "Case registration", enter the name of the sample you wish to create, and click "OK" to create the sample data.
-
-



-

- For Enterprise plan users
  - At the last step of new registration, you will be asked for an activation code.
  - Please follow the steps below to register.

Steps 1) through 6) are the same.	
• 7) Enter the activation code issued for each medical institution. • Please contact the administrator of your institution for the activation code. • If you do not know the administrator of your institution, please contact us. • • •	
• 8) Once the activation code is correctly entered, the app will be available for use.	

- Contact Us
  - inquiry-product@carbgem.com
  - Hours: Weekdays from 10:00 to 18:00 (Japan time)
  - Closed: Saturdays, Sundays, national holidays, and year-end and New Year holidays (Japan time)

- Reference: Classification of urine specimens
  - Classification 1: ClassU1
  - Classification 2: ClassU2

Class U1	Class U2
yeast	*Candida* spp.
GPC	GPC cluster
GPC	*Enterococcus faecalis*
GPC	*Enterococcus faecium*
GPC	*Streptococcus agalactiae*
GPC	other GPC
GPR	*Corynebacterium* spp.
GNR	*Enterobacter cloacae*
GNR	*Escherichia coli*
GNR	*Klebsiella oxytoca*
GNR	*Klebsiella pneumoniae*
GNR	*Pseudomonas aeruginosa*
GNR	other GNR Enterobacteriaceae
GNR	other GNR Glucose non-fermenting bacteria
GNC	GNC
Poly.	polymicrobial
None	No bacteria.