# Application of Microbial and Parasitology Techniques for Diagnosis in Laboratory Rodents

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Pathogen diagnosis in rodents through effective health monitoring programs is essential for maintaining high welfare and standards in laboratory animal facilities. Although many animal facilities are increasingly adopting real-time polymerase chain reaction testing of environmental or filter samples for pathogen detection, thereby reducing the need for sentinel animals, classical methods such as microbiological assays and microscopic examination for parasites, remain essential and relevant tools in laboratory animal diagnostics. This article describes protocols for the identification of bacterial, viral, and parasitic agents in mice and rats in accordance with the recommendations of the Federation of European Laboratory Animal Science Associations. The methodologies presented detailed procedures for sample collection from mice and rats and for screening these samples using bacterial, parasitological, and viral panels. In addition, we describe bacterial culture techniques and the use of selective and differential media for pathogen isolation, as well as step-by-step protocols for the detection of pinworms, mites, and parasite eggs. These protocols provide a practical foundation for establishing a basic diagnostic laboratory to support standardized animal husbandry and health monitoring quality. © 2026 The Author(s). *Current Protocols* published by Wiley Periodicals LLC.

**Basic Protocol 1**: Gathering rodent samples for pathogen screening
**Basic Protocol 2**: Inoculation and screening of bacterial cultures from rodent samples
**Support Protocol 1**: Identification of bacteria using differential media
**Basic Protocol 3**: Identification of *Helicobacter* by PCR targeting the 16S rRNA gene
**Basic Protocol 4**: Pinworm screening by microscopy
**Support Protocol 2**: Confirmation and specification of parasites (pinworms and mites) by PCR
**Basic Protocol 5**: Mite screening by microscopy
**Basic Protocol 6**: Virus identification by serology

Keywords: animal facility • microbiology methods • parasites • rodents



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## INTRODUCTION

The presence of viral, bacterial, and parasitic infections can confound the interpretation of research findings (Treuting et al., 2012); therefore, health monitoring and continuous diagnosis of infectious pathogens in research animals and breeding colonies are essential for assessing infection prevalence and maintaining appropriate sanitary and environmental conditions within animal facilities (Pritchett-Corning et al., 2009). Pathogen prevalence is highly associated with the status of the animal facility, i.e., the sanitary and hygienic conditions and the management and design of the facility infrastructure (Rapaport et al., 2025).

To monitor rodent colony health in research facilities, soiled-bedding sentinel animals have been traditionally used (Albers et al., 2023; Carriquiriborde et al., 2020; Mailhiot et al., 2020; Rapaport et al., 2025; Schlapp et al., 2018). The samples collected include nasopharynx swabs, blood, feces, and fur-skin. Whereas classical health surveillance relies on bacterial cultures, serological testing, and parasitological examinations, molecular diagnostic methods have been developed to enable precise identification of bacterial pathogens and pinworms.

These include real-time polymerase chain reaction (PCR), DNA sequencing, and 16S ribosomal DNA (rDNA) and 18S rRNA analyses. More recently, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has emerged as a reliable and efficient tool for the identification of anaerobic bacteria (Alcalá et al., 2021; Compton, 2020; Dubourg et al., 2018; Sastre et al., 2018).

Environmental monitoring within animal facilities is also an integral component of routine health surveillance and includes defined sampling frequencies, timing, duration, and sample sizes per surface area. Monitoring is performed using a range of techniques, such as contact and settle plates, surface swabbing, active air sampling, and ATP-based methods to detect the presence of viable or nonviable organic material (Albers et al., 2023; Ihssen et al., 2021; Kim et al., 2023; Lupini et al., 2022; Miller & Brielmeier, 2018; Turner et al., 2010).

The pathogens are listed according to the recommendations of the Federation of European Laboratory Animal Science Associations (FELASA; Laber et al., 2016; Mähler et al., 2014). However, because of the diversity of cultured animal samples, bacterial identification poses a challenge in creating a health report according to the FELASA recommendation.

Here, we describe multiple methods to identify and characterize putative pathogens in mice and rats housed in research animal facilities, integrating traditional culture-based microbiology, PCR-based assays, and direct microscopic examinations. Basic Protocol 1 describes methods for gathering samples from different organs of rodents. Basic Protocol 2 delineates the culture-based processing of these samples, specifying the agar plates and enrichment media used for bacterial screening. Support Protocol 1 describes the core bacterial identification workflow using differential media and biochemical assays to confirm

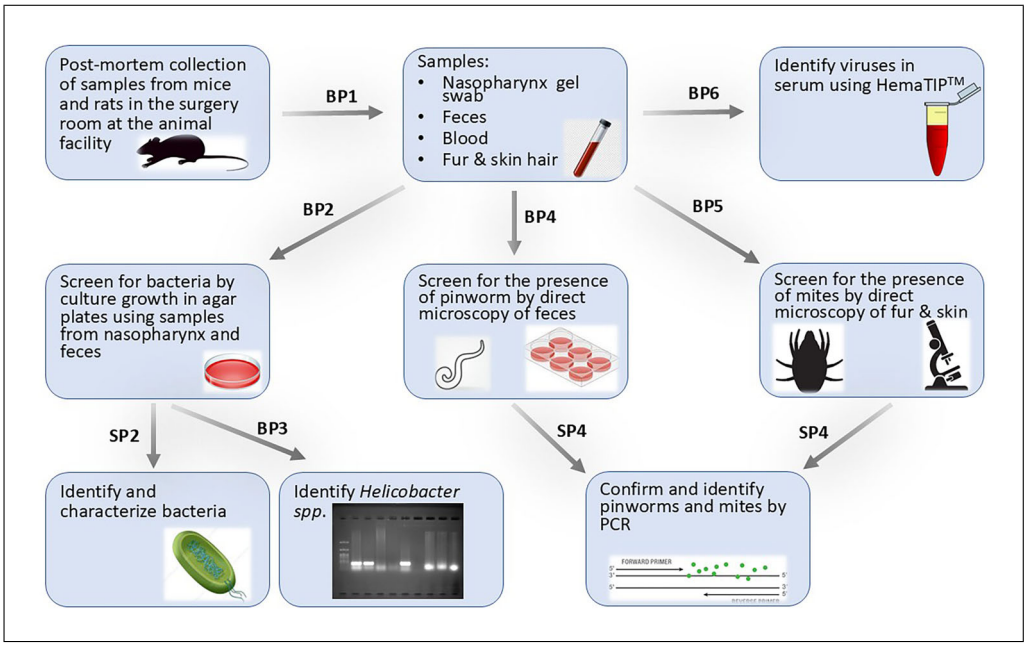



**Figure 1** Workflow steps described in the protocols. Basic Protocol 1 covers sample collection from rodent organs. Basic Protocol 2 details culture-based sample processing. Support Protocol 1 is a bacterial identification workflow. Basic Protocol 3 presents the PCR-based framework for detection of *Helicobacter* spp. Basic Protocols 4 and 5 describe direct microscopic examination for the identification of nematodes and mites, respectively. Support Protocols 4 and 5 outline the PCR-based strategies for parasite species identification. Basic Protocol 6 summarizes viral serology testing. Abbreviations: BP, Basic Protocol; PCR, polymerase chain reaction; SP, Support Protocol.

or exclude the presence of specific pathogens. Basic Protocol 3 provides the framework of *Helicobacter* spp. identification. Basic Protocols 4 and 5 describe direct microscopic examination for identifying nematodes and mites, respectively. PCR-based strategies for identifying parasite species are outlined in Support Protocol 2. Finally, Basic Protocol 6 briefly describes viral serology testing performed by external diagnostic laboratories. Figure 1 illustrates the workflow of the protocols described in the paper.

*NOTE*: All protocols involving animals must be reviewed and approved by the appropriate Animal Care and Use Committee and must follow regulations for the care and use of laboratory animals. Appropriate informed consent is necessary for obtaining and use of human study material.

## GATHERING RODENT SAMPLES FOR PATHOGEN SCREENING

***BASIC PROTOCOL 1***

Specimens from mice and rats, including sentinels from the health monitoring program, should be collected postmortem under sterile conditions.

### *Materials*

Minitip rayon swab with firm aluminum wire applicator and Amies agar gel medium (Transystem Traditional Bacteriology Transport, cat. no. 110C, Copan Diagnostics, Murrieta, CA, USA)

HemaTIP Microsampler (Charles River Laboratories, Wilmington, MA, USA) or equivalent

Rayon dry plastic swab with regular tip (e.g., CLASSIQSwabs Specimen Collection and Transport System Sterile, cat. no. 155C, Copan Diagnostics, Murrieta, CA, USA)

Sterile saline solution

1.5-ml microcentrifuge tubes
6-well plates
Microscope slides
Forceps

1. Collect swab samples from the animal's nasopharynx using the Transystem Traditional Bacteriology Transport minitip rayon swab. The swab will be used for inoculation of bacterial cultures (see Basic Protocol 2).
2. Collect blood by salivary gland punctation using a HemaTIP Microsampler or equivalent. The HemaTIP is shipped to Charles River Research Animal Diagnostic Services for serology assessment (see Basic Protocol 6).
3. Collect fecal samples/pellets from the duodenum, cecum, and colon.

   *The fecal samples/pellets can be combined after collection and further processed together.*

4. Distribute the fecal samples as follows:
   a. Microcentrifuge tube for microbiology culture using a plastic rayon swab for feces inoculation (see Basic Protocol 2, Basic Protocol 3, and Support Protocol 1);
   b. 6-well plate (pre-prepared with 3 ml saline/each well) for pinworm screening under microscope (see Basic Protocol 4).
5. Collect fur hair with forceps and mount on a microscope slide. The fur hair will be used in screening for ectoparasites and mites (see Basic Protocol 5).

## *BASIC PROTOCOL 2* INOCULATION AND SCREENING OF BACTERIAL CULTURES FROM RODENT SAMPLES

The prevalence of bacterial pathogens in various rodent facilities has been documented over the years. For example, *Staphylococcus aureus* is the most common pathogen recovered from skin lesions in laboratory mice (Battaglia & Garrett-Sinha, 2023); therefore, it is not routinely excluded from specific-pathogen-free (SPF) barrier rooms. Because most laboratories use SPF mice for their experiments, natural *S. aureus* colonization of experimental mice might be far more common than expected (Schulz et al., 2017).

The Gram-negative bacteria *Pasteurella pneumotropica* (included in FELASA panels), *Proteus* spp., and *Enterobacter cloacae* (the latter two are not included in the FELASA recommendations) were the bacteria most commonly isolated in several studies (Albers et al., 2023; Benga et al., 2018; McInnes et al., 2011; Rapaport et al., 2025). All three pathogens can be isolated by classical microbiology, as described below.

### Laboratory Infrastructure

Bacterial culture screening is the core of health monitoring in the animal facility. A laboratory performing routine microbiological testing should be equipped with a class II biological safety cabinet (BSC), a 37°C, 5% $CO_2$ incubator, a polymerase chain reaction (PCR) machine, an inverted microscope, a visible-light microscope, digital cameras attached to the microscopes, an agarose gel electrophoresis apparatus, and an ultraviolet (UV) light table and imaging system attached to the microscope for imaging captures.

*CAUTION*: The isolation and identification of bacteria, including the systematic inoculation of nasopharynx gel swab and fecal specimens in agar plates, should be performed in a BSC under Biosafety Level 2 (BSL-2; CDC Laboratories, 2020; Rapaport, 2021). Personal protective equipment—gloves, mask, and lab coat—must be worn while working in the BSC.

**Table 1** Summary of Sample Collection and Techniques for Pathogen Identification in Laboratory Animals

| Samples and assays | Blood/serum virology | Nasopharynx gel swab bacteriology | Feces bacteriology | Feces parasitology | Environmental cotton swab + feces bacteriology and parasitology | Fur-skin parasitology |
|---|---|---|---|---|---|---|
| **Techniques or assays** | Multiplex fluorescence immunoassay, immunofluorescence assay | Culture | Culture | Culture, direct microscopy | Polymerase chain reaction | Direct microscopy |
| **Materials: plates and media** | – | Tryptic soy agar containing defibrinated sheep blood plates, MacConkey agar | MacConkey agar, cetrimide agar, selenite broth | – | – | – |
| **Biochemical tests** | – | Enterotests: indole, *ortho*-nitrophenyl-β-galactoside (ONPG), glucose, hydrogen sulfide, motility, urea | | – | – | – |
| **Selective media** | – | CHROMagar orientation, CHROMagar Staph aureus, CHROMagar Salmonella Plus, XLD agar | | – | – | – |
| **Enzymatic tests** | – | Oxidase, coagulase | | – | – | – |

The samples collected according to Basic Protocol 1 are processed using standard protocols for the detection of viruses, parasites, and bacteria. Bacterial detection is performed using selective agar plates, followed by biochemical and enzymatic tests, as summarized in Table 1.

Table 2 lists the infectious agents tested as part of routine health monitoring of mice and rats in animal facilities in accordance with FELASA recommendations. The monitoring program includes virological, bacteriological and parasitological screening. Additional bacterial species not explicitly listed in the FELASA-recommended panels may also be detected using the same diagnostic protocols.

Figure 2 illustrates the procedures for sample inoculation in agar plates and tubes.

### *Materials*

Nasopharygeal gel swab samples and fecal samples (Basic Protocol 1)
Tryptic soy agar (TSA) + defibrinated sheep blood plates (cat. no. PD049, Hylabs, Rehovot, Israel)
MacConkey agar plates (cat. no. PD032, Hylabs, Rehovot, Israel)
Cetrimide agar plates (cat. no. PD210, Hylabs, Rehovot, Israel)
Selenite broth medium (cat. no. tT130, Hylabs, Rehovot, Israel)

QuadLoop Needle & Sphere end (cat. no. 815-011-20-001, Miniplast, Caesarea, Israel)
37°C, 5% $CO_2$ incubator

**Table 2** Agents Tested in Rodents During Animal Facility Health Monitoring

| Test | Pathogen | Material |
|---|---|---|
| Virology/serology panel, mouse (FELASA panel) | Mouse hepatitis virus, mouse rotavirus, minute virus of mice, mouse parvovirus, pneumonia virus of mice, Sendai virus, Theiler's murine encephalomyelitis virus, ectromelia virus, lymphocytic choriomeningitis virus, mouse adenovirus type 1, 2, mouse cytomegalovirus, reovirus type 3, generic parvovirus, murine norovirus, *Mycoplasma pulmonis*, *Clostridium piliforme* | Mouse blood |
| Virology/serology panel, rat (FELASA panel) | Zoonotic hantaan virus, Toolan's H1-rat parvovirus, rodent adenovirus strain 1, 2, rat parvovirus, rat minute virus, Kilham's rat virus-parvovirus, rodent pneumovirus, rat coronavirus, rodent reovirus, rat theilovirus, *Pneumocystis carinii*, rat cytomegalovirus, cilia-associated respiratory bacillus. | Rat blood |
| Microbiology (FELASA panel) | *Mycoplasma pulmonis*, *Bordetella bronchiseptica*, *Citrobacter rodentium*, *Clostridium piliforme*, *Corynebacterium kutcheri*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Pasteurella* spp., *Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococcus aureus*, β-hemolytic streptococci, *Streptococcus pneumonia*, *Helicobacter* spp., *Streptobacillus moniliformis*, dermatophytes, *Corynebacterium bovis*, *Pneumocystis carinii* | Nasopharynx and feces swabs |
| Microbiology, other isolated bacteria not listed in FELASA panel | *Proteus mirabilis*, *Proteus vulgaris*, *Morganela morganii*, *Staphylococcus saprophyticus*, *Staphylococcus epidermis*, *Providencia rettgeri*, *Bacillus* spp., *Pseudomonas stutzeri*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *Escherichia coli* "Shigella-like," *Enterococcus faecalis*, *Serratia marcescens*, *Staphylococcus xylosus*, *Mycoplasma genus* | Nasopharynx and feces swabs |
| Parasitology, pinworms | *Aspiculuris tetraptera*, *Syphacia muris* (rat), *Syphacia obvelata* | Feces |
| Parasitology, fur mites | *Myobia musculi*, *Radfordia affinis*, *Radfordia ensifera*, *Myocoptes musculinus* | Fur hair tape |
| Parasitology, protozoa, non-pathogenic | Chilomastix, Entamoeba, trichomonads | Feces |

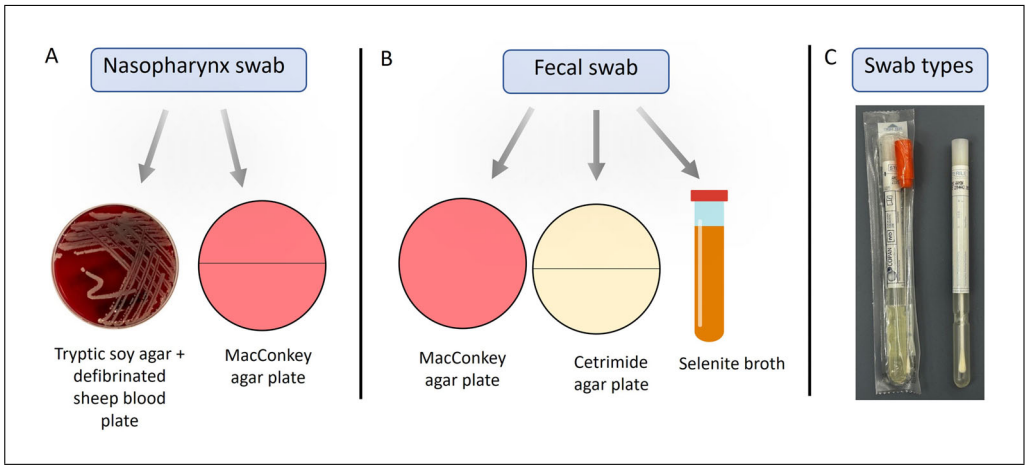



**Figure 2** Inoculation procedure following sample collection. (**A**) Inoculate tryptic soy agar containing defibrinated sheep blood plates (TSA blood) and MacConkey agar plates (use half a plate) with nasopharyngeal samples collected with a Transystem Traditional Bacteriology Transport minitip rayon swab. (**B**) Inoculate MacConkey agar plates, cetrimide agar plates (use half a plate), and selenite broth medium with fecal samples collected with plastic rayon swab. (**C**) Nasopharyngeal samples collected with TransystemTraditional Bacteriology Transport minitip rayon swab (left) and plastic rayon swab (right).

1. Using the nasopharyngeal gel swab, inoculate a TSA plate containing defibrinated sheep blood by streaking and spreading the sample across the agar surface. Distribute the inoculum evenly in a top-to-bottom or side-to-side motion to ensure full surface coverage (Fig. 2A).

   *Blood agar, a differential medium, is used to distinguish bacterial species based on hemolytic activity mediated by bacterial enzymes such as hemolysins.*

2. With the same nasopharyngeal gel swab, inoculate one half of a MacConkey agar plate (Fig. 2A).

   *Using one half of the MacConkey agar plate is sufficient; the other half can be used for another sample or left empty.*

3. Inoculate a MacConkey agar plate and one half of a cetrimide agar plate with the fecal material collected with the rayon dry plastic swab (see Basic Protocol 1); then cut the swab and place it in the selenite enrichment broth (Fig. 2B).

   *The cetrimide plate should be densely smeared with the fecal sample. Using one half of the cetrimide plate is sufficient; the other half can be used for another sample or left empty.*

4. Incubate all agar plates in an inverted position and all broth tubes overnight in a 37°C, 5% $CO_2$ incubator.

   *After incubation, the culture plates typically exhibit bacterial colony growth with varying morphology, including differences in color, size, and odor.*

5. Discard plates showing no bacterial growth according to standard laboratory procedures.

6. Test all colonies observed on the culture plates using Support Protocol 1.

## IDENTIFICATION OF BACTERIA USING DIFFERENTIAL MEDIA

***SUPPORT PROTOCOL 1***

This protocol involves the isolation of individual bacterial colonies and their identification in accordance with FELASA recommendations for identifying bacterial agents, as well as the identification of additional bacterial species not listed in the FELASA checklist (Table 2). Individual colonies from nasopharyngeal and fecal inoculated swabs that grew on the TSA plate containing defibrinated sheep blood, MacConkey agar plates, and cetrimide plates and/or in selenite broth (see Basic Protocol 2) are subjected to further characterization. Table 3 summarizes the culture media and diagnostic assays applied for the isolation, differentiation, and identification of bacterial pathogens as part of routine health monitoring in mice and rats. Selective and differential agar media, chromogenic plates, enrichment broths, biochemical tests, and Gram staining are used in a stepwise workflow to support identification of both Gram-positive and Gram-negative bacteria, in accordance with FELASA-based diagnostic protocols (Perry, 2017).

### *Materials*

Samples (nasopharyngeal and fecal swabs) collected from laboratory rodents (Basic Protocol 1)
Tryptic soy agar (TSA) + defibrinated sheep blood plates (cat. no. PD049, Hylabs, Rehovot, Israel)
MacConkey agar plates (cat. no. PD032, Hylabs, Rehovot, Israel)
Cetrimide agar plates (cat. no. PD210, Hylabs, Rehovot, Israel)
Selenite broth medium (cat. no. TT130, Hylabs, Rehovot, Israel)
CHROMagar orientation plates (cat. no. PD165, Hylabs, Rehovot, Israel)
Enterobacteriaceae differential medium (HY-Enterotest, cat. no. TT146, Hylabs, Rehovot, Israel)
Simmons citrate agar tube (cat. no. TT134, Hylabs, Rehovot, Israel)
Urea Agar Slant Tube (cat. no. R42, Hardy Diagnostics, Santa Maria, CA, USA)

**Table 3** Differential, Selective, and Biochemical Culture Media and Laboratory Tests Used for Identifying Bacteria in Laboratory Rodents

| Medium or test | Purpose and/or target organisms | Principle/selectivity | Interpretation/typical results |
|---|---|---|---|
| **Selenite broth** | Enrichment for *Salmonella* spp. | Inhibits growth of Gram-positive and many Gram-negative bacteria; *Salmonella* spp. are unaffected. | Clear or turbid broth. Turbidity indicates bacterial growth and requires further confirmatory testing. |
| **Cetrimide agar** | Selective and differential medium for *Pseudomonas aeruginosa* | Cetrimide (cetyltrimethylammonium bromide) inhibits most bacterial growth. When in contact with bacteria, it causes nitrogen and phosphorus release and promotes the production of fluorescein and pyocyanin pigments. | *P. aeruginosa* colonies are fluorescent under UV light (Fig. 5). Some enterics occasionally show slight yellowing of medium. Non-glucose-fermenting species may grow but the resulting off-white colonies are easily distinguishable from fluorescein-positive colonies. |
| **CHROMagar orientation** | Nonselective chromogenic medium for differentiation of common pathogens | Chromogenic substrates are cleaved by species-specific enzymes, producing characteristic colony colors. | *Escherichia coli*: dark pink to reddish<br>*Enterococcus*: turquoise blue<br>*Proteus* spp.: brown halo<br>*Klebsiella* spp., *Enterobacter*, *Serratia*: metallic blue<br>*Staphylococcus aureus*: small golden, opaque<br>*Citrobacter*: metallic blue with red halo<br>*Staphylococcus saprophyticus*: small, pink, opaque<br>*P aeruginosa*: translucent cream to blue |
| **Enterotest** | Identification of Enterobacteriaceae | Combined biochemical tube assessing indole production, *o*-nitrophenyl-β-galactoside (ONPG) hydrolysis, glucose fermentation (with and without $CO_2$ production), $H_2S$ production, motility, and urea hydrolysis.<br>The method is based on Kigler iron agar and triple iron sugar iron agar slants (Skillern & Overman, 1983). | The results are interpreted as a biochemical profile, enabling species-level identification. |
| **Simmons citrate agar** | Differentiation of Enterobacteriaceae | Tests bacteria's ability to use citrate as sole carbon source; citrate utilization produces alkaline byproducts that change the color of the bromothymol blue indicator. | A positive result is indicated by a color change from green to blue. |
| **Urea agar** | Detection of urease-producing bacteria | Urease hydrolyzes urea to ammonia and $CO_2$, increasing pH and changing the color of the phenol red indicator. | A positive result is indicated by a color change from light orange to pink/magenta. |

(*Continued*)

**Table 3** Differential, Selective, and Biochemical Culture Media and Laboratory Tests Used for Identifying Bacteria in Laboratory Rodents, *continued*

| Medium or test | Purpose and/or target organisms | Principle/selectivity | Interpretation/typical results |
|---|---|---|---|
| **Gram staining** | Primary bacterial classification | Differential staining based on cell wall structure (Paray et al., 2023) | Differentiates between Gram-positive and Gram-negative bacteria. |
| **Optochin test** | Differentiation of *Streptococcus pneumoniae* | Differentiates *S. pneumoniae* from other α-hemolytic streptococci based on its sensitivity to optochin, which inhibits bacterial ATP synthase, producing a clear zone of inhibition around the disc. | If no bacteria grow around the optochin disc, the culture is presumed to be *S. pneumoniae*. |
| **Coagulase assay** | Differentiation of *S. aureus* from other *Staphylococcus* species | Detects the ability of certain bacteria to produce coagulase, which converts fibrinogen to fibrin, producing visible agglutination (slide test) or clot formation (tube test). | Agglutination (slide test) or clot formation (tube test) indicates a positive result. |
| **CHROMagar Staph aureus** | Identification of *S. aureus* | Chromogenic substrates are cleaved by *S. aureus* enzymes, producing characteristic colony colors | Mauve/pink-purple colonies indicate the presence of *S. aureus*. |
| **CHROMagar Salmonella/XLD** | Identification of *Salmonella*, including lactose-positive *Salmonella*, *Salmonella typhi*, and *Salmonella typhimurium* | CHROMagar Salmonella identifies *Salmonella* by species-specific enzymatic cleavage of chromogenic substrates producing characteristic colony colors; XLD agar differentiates *Salmonella* based on xylose fermentation, lysine decarboxylation, and hydrogen sulfide production. | Pink-mauve colonies on the CHROMagar *Salmonella* side indicate the presence of *Salmonella* species. Other microorganisms are inhibited or form colorless or turquoise colonies.<br>Pink-red colonies with black centers, pink-yellow colonies with black centers, or yellow colonies with black centers on the XLD side indicate the presence of *Salmonella* species. |
| **Oxidase strip** | Differentiation of Gram-negative bacteria | The oxidase strip test detects cytochrome *c* oxidase from the oxidation of a colorless reagent to a purple compound. | A rapid purple color indicates an oxidase-positive organism (*P. aeruginosa*, *Neisseria*, *Vibrio*). Oxidase-negative organisms (no color) include Enterobacteriaceae (*E. coli*, *Klebsiella*, *Salmonella*). |

Optochin antibiotic disc (cat. no. DD009, Hylabs, Rehovot, Israel)
CHROMagar Staph aureus plates (cat. no. PD235, Hylabs, Rehovot, Israel)
HY-Oxidase-impregnated paper test strips (cat. no. IS002, Hylabs, Rehovot, Israel)
CHROMagar Salmonella Plus/XLD Agar plates (cat. no. DD071, Hylabs, Rehovot, Israel)
Staph Rapid Latex Test Kit (cat. no. 271060, Hylabs, Rehovot, Israel)
Gram Stain Set (cat. no. KI5010, Hylabs, Rehovot, Israel)

QuadLoop Needle & Sphere end (cat. no. 815-011-20-001, Miniplast, Caesarea, Israel)
UV light table and imaging system

### *Identification of Gram-positive and Gram-negative bacteria*

This protocol uses several laboratory tests to identify Gram-positive (*Streptococcus* spp., *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Micrococcus*, *Bacillus cereus*, *Lactinobacillus muris*, and *Corynebacterium bovis*; Fig. 3) and Gram-negative bacteria (*Klebsiella pneumonia*, *Klebsiella oxytoca*, *Pasteurella* spp., *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*; Fig. 4).

1. Inoculate TSA plates with defibrinated sheep blood and MacConkey agar plates under aseptic conditions. Incubate the blood agar plates under standard microbiological conditions. Examine colony growth (see Basic Protocol 2).

   *Only Gram-negative bacteria will grow on MacConkey agar plates*

2. Assess colony morphology on MacConkey agar plates and TSA plates with defibrinated sheep blood, including colony size, shape, and hemolytic pattern after overnight incubation in the incubator.

3. Subculture selected colonies from TSA and MacConkey agar plates in CHROMagar Orientation plates to aid preliminary differentiation (see Table 3 for interpretation of colony color).

   *Make sure that only a single colony is picked for each inoculation.*

4. Perform biochemical testing using the following media picking a colony from TSA and MacConkey agar plates:
   • Enterotest (indole-containing tube);
   • Simmons citrate agar;
   • Urea agar.

   *The results are interpreted as a biochemical profile enabling species-level identification.*

### *Identification of Gram-positive bacteria*

5. Evaluate isolates suspected to be *Streptococcus* spp. by performing an optochin susceptibility test, which is used to differentiate *Streptococcus* pneumoniae from other α-hemolytic streptococci according to the growth around the optochin disc (Ercibengoa, 2021).

   *Optochin inhibits Streptococcus pneumoniae growth; therefore, growth around the optochin disc indicates the presence of another Streptococcus spp.*

6. Evaluate isolates suspected to be *Staphylococcus aureus* by inoculating them on CHROMagar Staph aureus plates and assessing colony color.

   *Rose-colored colonies are indicative of Staphylococcus aureus (positive), whereas blue colonies are negative for this bacterium.*

7. Confirm that the isolates are *Staphylococcus aureus* by performing an enzymatic coagulase test using the Staph Rapid Latex Test Kit, using a colony of *E. coli* as a negative control.

### *Identification of Gram-negative bacteria*

8. Evaluate isolates suspected to be *Klebsiella pneumonia* or *Klebsiella oxytoca* on CHROMagar Salmonella Plus XLD plates using *E. coli* as control.

   *Yellow agar and colonies on the XLD agar side indicate the presence of Klebsiella. Blue colonies on the Salmonella Plus side indicate the presence of E. coli.*

9. Evaluate isolates suspected to be *Proteus mirabilis* on CHROMagar Salmonella Plus XLD plates.

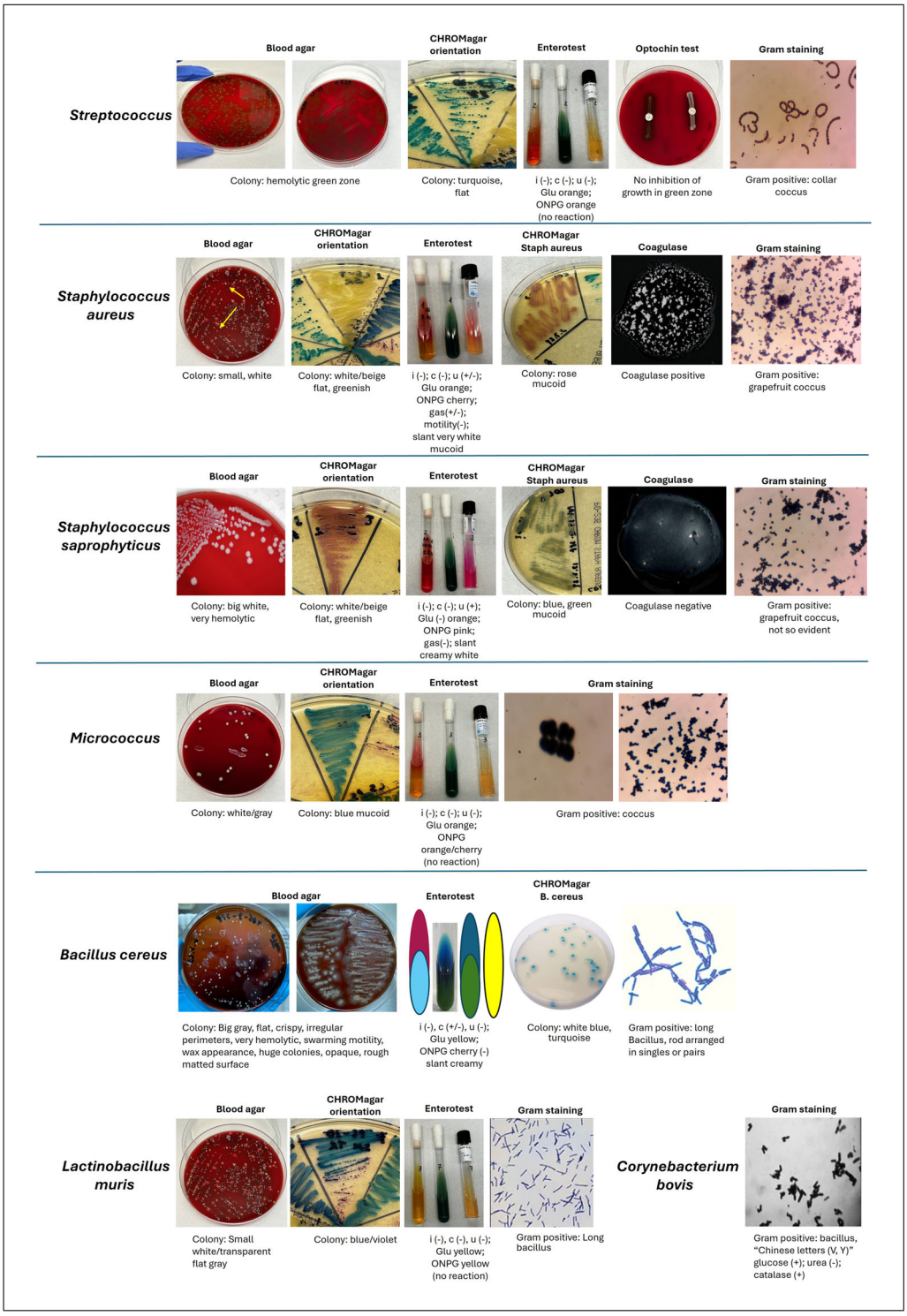



**Figure 3** Phenotypic characterization of Gram-positive bacteria isolated from plate colonies. Representative images showing test results to identify the Gram-positive bacteria *Streptococcus* spp., *Staphylococcus aureus*, *Staphylococcus saprophyticus, Micrococcus, Bacillus cereus, Lactinobacillus muris*, and *Corynebacterium bovis*, as described in Support Protocol 1. Abbreviations: i, indole (a colorless cap indicates a negative reaction, a pink cap indicates a positive reaction); c, citrate (green indicates a negative reaction, blue indicates a positive reaction); u, urea (yellow indicates a negative reaction, pink/rose indicates a positive reaction); Glu, glucose (orange indicates a negative reaction, yellow indicates a positive reaction); ONPG, *o*-nitrophenyl-β-galactoside (cherry indicates a negative reaction, orange indicates a positive reaction); gas, presence of bubbles in the medium due to $CO_2/H_2$ production from carbohydrate fermentation; (+), positive reaction; (-), negative reaction. Arrows indicate the denoted bacterial colony.


**Figure 4** Phenotypic characterization of Gram-negative bacteria isolated from plate colonies. Representative images showing test results to identify the Gram-negative bacteria *Klebsiella pneumonia, Klebsiella oxytoca, Pasteurella* spp., *Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa*, and *Escherichia coli*, as described in Support Protocol 1. Abbreviations: i, indole (a colorless cap indicates a negative reaction, a pink cap indicates a positive reaction); c, citrate (green indicates a negative reaction, blue indicates a positive reaction); u, urea (yellow indicates a negative reaction, pink/rose indicates a positive reaction); Glu, glucose (orange indicates a negative reaction, yellow indicates a positive reaction); ONPG, *o*-nitrophenyl-β-galactoside (cherry indicates a negative reaction, orange indicates a positive reaction); gas, presence of bubbles in the medium due to $CO_2/H_2$ production from carbohydrate fermentation; (+), positive reaction; (-), negative reaction. Arrows indicate the denoted bacteria colony.

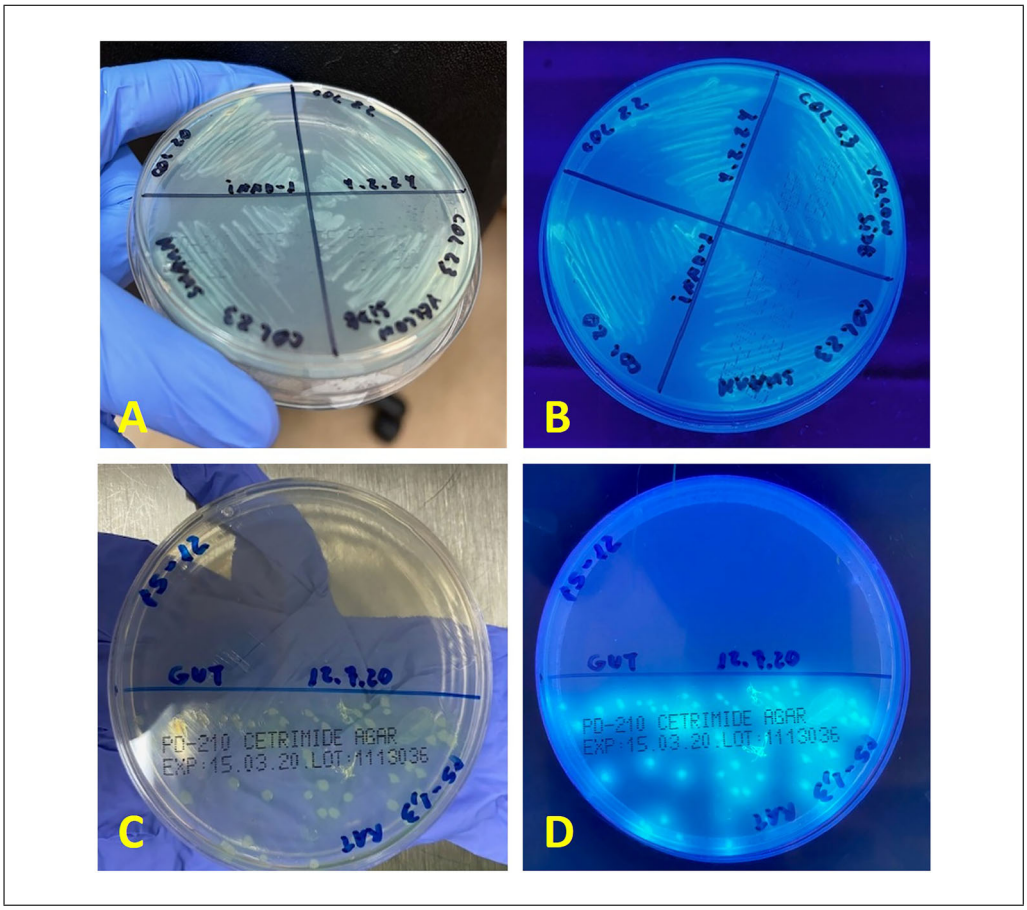



**Figure 5** Cetrimide plates for identification of *Pseudomonas aeruginosa*. (**A**, **C**) Under visible light; (**B**, **D**) under UV light, revealing fluorescent colonies. The combination of pyocyanin production and ability to grow at 4°C is sufficient to distinguish *P. aeruginosa* from other *Pseudomonas* species. Check fluorescence under UV light after 24 and 48 hr, as sometimes growing the culture overnight is not sufficient to observe the fluorescence effect.

*Red agar and pink to orange colonies, sometimes with a black center on the XLD agar side indicate the presence of Proteus mirabilis. Sometimes small yellowish colonies grow on the Salmonella Plus side.*

10. *Pasteurella pneumotropica* grows as gray colonies on blood agar, whereas other Pasteurellaceae species appear as yellow lytic colonies (Dafni et al., 2019). Evaluate isolates suspected to be *Pasteurella* spp. using HY-Oxidase-impregnated paper strips.

    *HY-Oxidase-impregnated paper strips will turn blue in the presence of Pasteurella spp.*

11. Evaluate isolates suspected to be *Pseudomonas aeruginosa* on cetrimide plates incubated at 42°C for 24-48 hr. Examine growth under UV light after 24 and 48 hr.

    *The combination of pyocyanin production and ability to grow at 4°C is sufficient to distinguish P. aeruginosa from other Pseudomonas species. The colonies emit fluorescence under UV light.*

12. Perform Gram staining on all isolates to confirm Gram-positive versus Gram negative bacterial morphology and status.

## PCR DETECTION OF *HELICOBACTER* TARGETING THE 16S rRNA GENE

*Helicobacter* species—including *Helicobacter hepaticus* and *Helicobacter bilis*, which are included in the FELASA-recommended monitoring panel—are commonly detected

in laboratory mouse feces (Butt et al., 2023; Mailhiot et al., 2020; Whary & Fox, 2006). PCR is the standard method for their identification (Riley et al., 1996). High-throughput multiplex assays, developed as sensitive DNA-based screening tools for routine health monitoring of laboratory animals, also enable efficient detection of *Helicobacter* spp. (Butt et al., 2023).

### *Materials*

Rodent fecal samples (Basic Protocol 1)

QIAamp fast DNA stool kit (cat. no. 51604, Qiagen, Redwood City, CA, USA)
PCR primers targeting the 16S rRNA gene (Riley et al., 1996):
Forward primer: 5′-CTATGACGGGTATCCGGC-3′
Reverse primer: 5′-ATTCCACCTACCTCTCCCA-3′
Hy-Taq Ready Mix (cat. no. EZ-3006, Hylabs, Rehovot, Israel)
Ethidium Bromide Dropper, 0.5 µg/ml (cat. no. BP717/BP451, Hylabs, Rehovot, Israel)
1% (w/v) agarose gel
100 bp DNA Ladder Ready-to-Use (cat. no. DMO001-R500, GeneDirex Inc., Las Vegas, NV, USA)

2-ml microcentrifuge tubes
PCR SimpliAmp Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA)

1. Place 6-8 pellets of feces from mouse or half a pellet of feces from rat in a 2-ml microcentrifuge tube and keep on ice. Extract DNA from feces pellets by using QIAamp fast DNA stool kit. Take 1 µl of DNA for the PCR reaction.
2. Prepare a PCR reaction mix using the forward and reverse primers targeting the 16S rRNA gene.
3. Load the PCR reactions into the thermal cycler.
4. Perform an initial denaturation for 5 min at 94°C.
5. Amplify the target sequence for 30 cycles under the following conditions:

   30 s 94°C (denaturation)
   45 s 60°C (annealing)
   1 min 72°C (extension).
6. Complete a final extension at 72°C for 1 min.
7. Hold the reactions at 10°C until further analysis.
8. Analyze the products on a 1% agarose gel.

   *The Helicobacter spp. band size is estimated at 400 bp (Fig. 6).*

***BASIC PROTOCOL 4***

## PINWORM SCREENING BY MICROSCOPY

The most common nematode infestations are *Syphacia obvelata* and *Aspiculuris tetraptera* (Abdel-Gaber et al., 2018; Ain-Fatin et al., 2022; Omer et al., 2020; Rapaport et al., 2025). These are transmitted through ventilation systems, breeding cages, and the hands of laboratory technical staff (Lytvynets et al., 2013).

Pinworms specimens are identified first by light microscopy, followed by PCR-based screening to confirm and specify the endoparasites (see Support Protocol 2). NGS has been to be effective in detecting pinworms and mites in SPF filters (Lupini et al., 2022) but can be confirmed also reported.

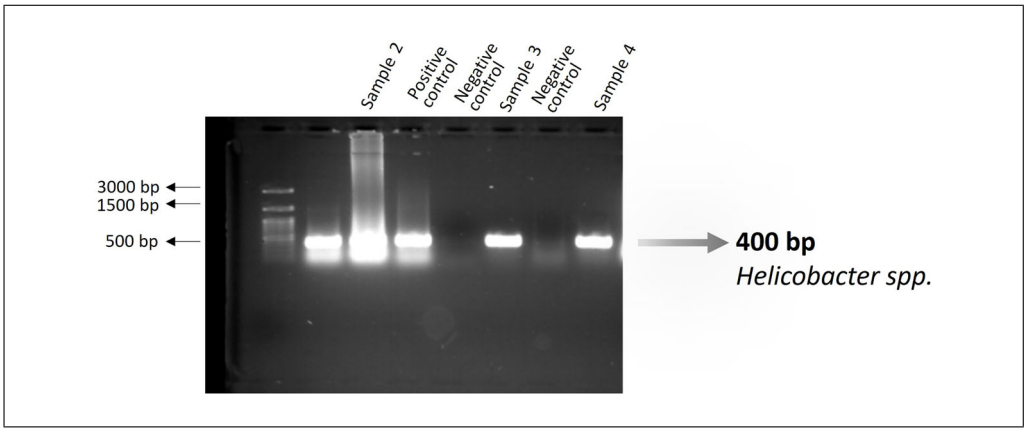



**Figure 6** Identification of PCR-amplified product for *Helicobacter* spp. in 1% agarose gel photographed under UV light. The *Helicobacter* spp. amplification band is at ~400 bp.

### *Materials*

Rodent gut contents, collected under aseptic conditions (Basic Protocol 1)
Sterile phosphate-buffered saline (PBS)

6-well plates
Nikon Eclipse Ts2 Inverted LED Phase Contrast Microscope (Nikon Instruments Inc., Melville, NY, USA)

1. Transfer the mouse or rat gut contents into individual wells of a 6-well plate containing 3 ml/well PBS.
2. Gently disperse the sample by squeezing and mixing to allow uniform suspension in PBS.
3. Inspect the suspension under an inverted microscope for the presence of parasite eggs and pinworms.
4. Identify the presence of eggs and pinworms and classify them based on their morphological characteristics (Abdel-Gaber, 2016; Figs. 7 and 8).

## PCR CONFIRMATION AND SPECIFICATION OF PARASITES (PINWORMS AND MITES)

***SUPPORT PROTOCOL 2***

Real-time PCR can be used to confirm the presence of endo- and ectoparasites. To that end, feces samples as well as swabs of cages are collected.

This Support Protocol also supports Basic Protocol 5.

### *Materials*

Fecal samples (Basic Protocol 1)
Microcentrifuge tubes
Rayon dry plastic swab with regular tip (CLASSIQSwabs Specimen Collection and Transport System Sterile, cat. no. 155C, Copan Diagnostics, Murrieta, CA, USA)

1. Place up to 10 fecal pellets per mouse or one fecal pellet per rat into a sterile microcentrifuge tube.
2. Collect environmental swabs from the interior surfaces of cages using a plastic rayon swab.
3. Collect skin swabs from fur-skin of mice or rats using a plastic rayon swab.

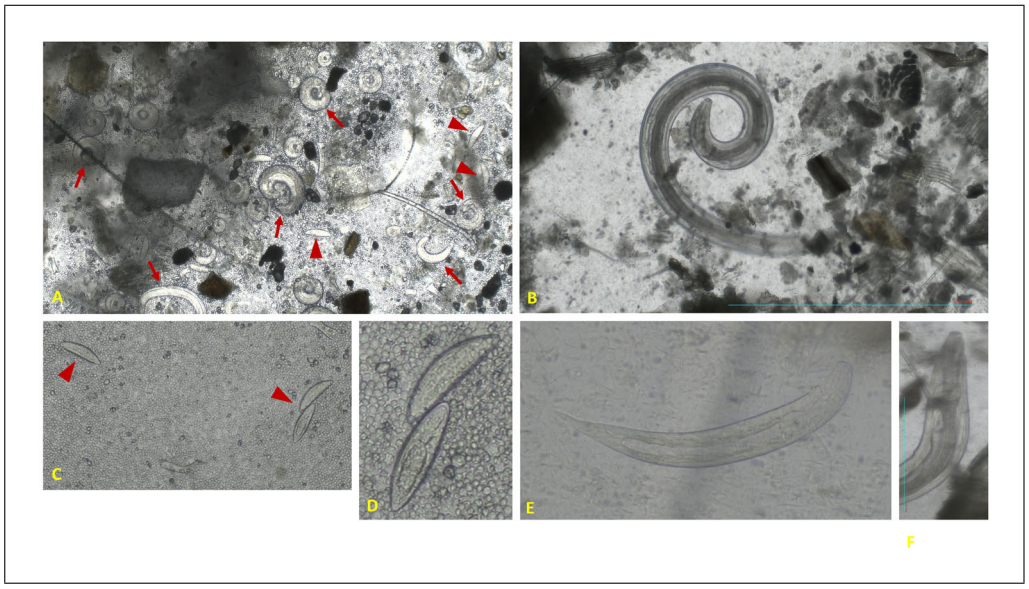



**Figure 7** Nematodes in mice feces observed by light microscopy. (**A**) Nematode-infested sample; (**B**) adult pinworm; (**C**, **D**) banana-shaped eggs from *Syphacia obvelata*; (**E**) young pinworm; (**F**) adult head nematode. Arrows, pinworms; arrowheads, eggs. Magnification: 40× (**A**, **B**, **C**, **F**), 200× (**E**).

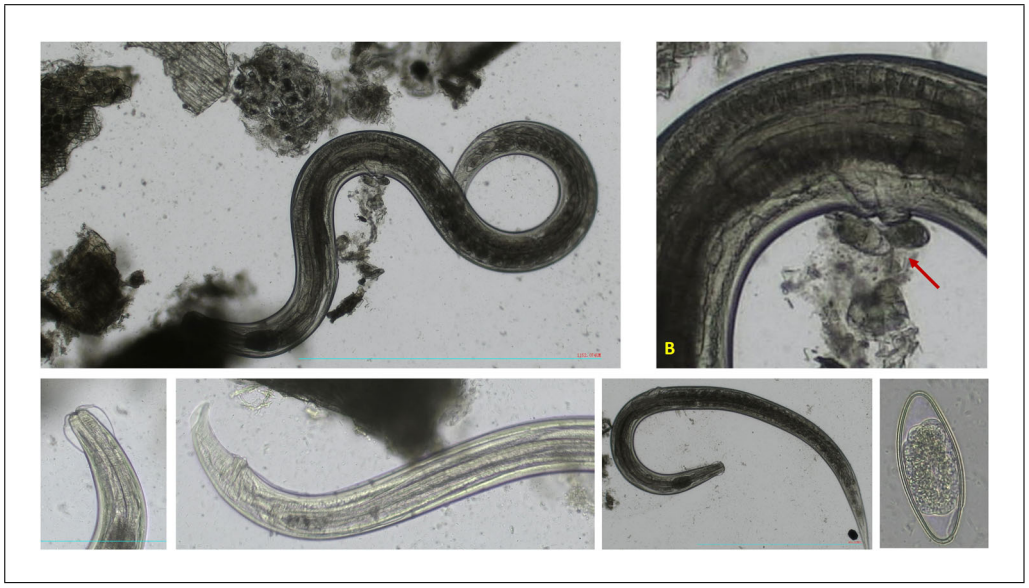



**Figure 8** *Aspiculuris tetraptera* in mice feces observed by light microscopy. (**A**) Gravid female laying eggs; (**B**) a magnified image of the eggs; (**C**) adult male head with halo; (**D**) spicule male tail; (**E**) adult female; (**F**) egg. Arrows point to eggs. Magnification: 40× (**A**–**E**), 200× (**F**).

4. Place the swabs into a sterile microcentrifuge tube and cut the excess swab shaft to allow secure closure of the tube.

5. Combine the fecal sample tube and the swab tube and ship the tube to Charles River Research Animal Diagnostic Services (Wilmington, MA, USA) for PCR-based analysis.

   *PCR testing can be used to detect intestinal nematodes and distinguish between Syphacia obvelata and Aspiculuris tetraptera. Targeted PCR assays for ectoparasite detection enable differentiation among Myobia musculi, Radfordia affinis, Radfordia ensifera, and Myocoptes musculinus (Table 2).*

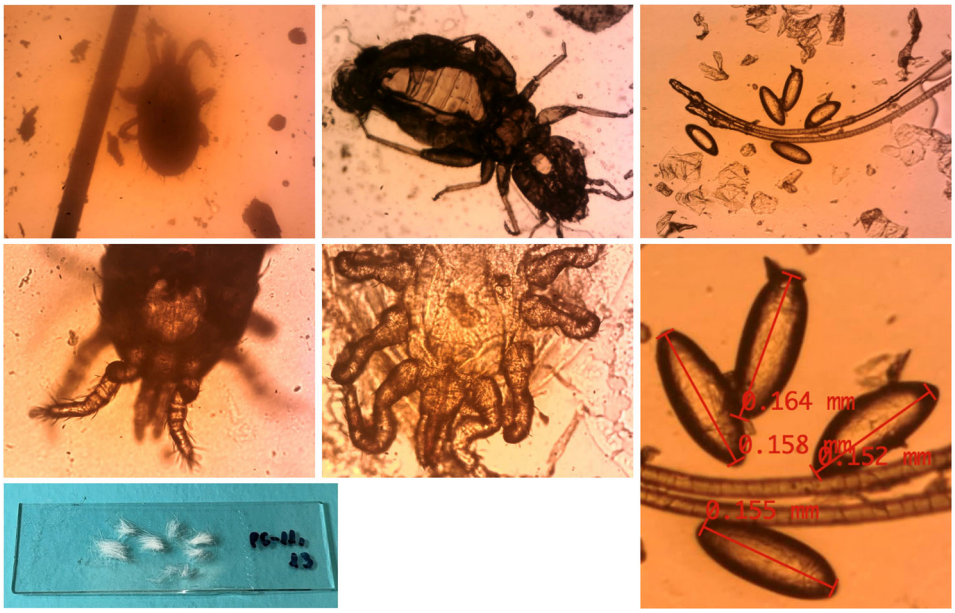



**Figure 9** Ectoparasites from fur-skin observed by light microscopy. (**A**, **B**, **D**, **E**) Different mites; (**C**) mite eggs; (**F**) egg lengths (0.155-0164 mm); (**G**) slide tape sample with fur-skin. Magnification: 40× (**A**, **D**, **E**), 100× (**B**), 200× (**C**), 400× (**F**).

## MITE SCREENING BY MICROSCOPY

***BASIC PROTOCOL 5***

Mite screening is performed using direct microscopic examination with the adhesive tape/fur-skin/hair method, followed by confirmatory PCR testing.

### *Materials*

Fur hair from mice or rats
Microscope slides
Microscope (Nikon Instruments Inc., Melville, NY, USA).

1. Place the fur hair on microscope slides.
2. Inspect the slide under an inverted microscope for the presence of mites and classify them based on their morphological characteristics (Whary et al., 2015; Fig. 9).

   *Please refer to Support Protocol 2 for information on confirming the presence of mites by PCR.*

## VIRUS IDENTIFICATION BY SEROLOGY

***BASIC PROTOCOL 6***

The serum of mice and rats is commonly tested for viral agents, with mouse norovirus, mouse hepatitis virus, rotavirus, and pneumonia virus being among the most prevalent (Albers et al., 2023; McInnes et al., 2011; Rapaport et al., 2025; Treuting et al., 2012). FELASA recommendations for virus panels are detailed in Table 2.

### *Materials*

HemaTIP Microsampler (Charles River Laboratories, Wilmington, MA, USA)

### *Methods*

1. Collect 20 µl of mouse or rat blood using a HemaTIP (see Basic Protocol 1).
2. Ship the collected blood samples to Charles River Research Animal Diagnostic Services (Wilmington, MA, USA) for analysis by multiplex fluorescence immunoassays.

## COMMENTARY

### Background Information

Soiled-bedding sentinel health monitoring relies on the transmission of the infectious agents from colony animals to sentinel animals, mainly through the transfer of soiled bedding.

Sentinel animals (mice and rats) are tested regularly using diagnostics based on bacterial cultures, serology, and direct microscopy. Although 16S ribosomal DNA sequencing is widely used to identify bacterial isolates in rodents (Benga et al., 2014), in recent years, PCR-based analysis of exhaust air dust samples (Gerwin et al., 2017; Mailhiot et al., 2020; Pettan-Brewer et al., 2020) and Pathobinder filters placed on cages have been suggested as alternative methods for pathogen detection in laboratory animals (Albers et al., 2023; Niimi et al., 2018; Winn et al., 2022). Metagenomic next-generation sequencing also enables monitoring of the murine colony microbiota and facilitates pathogen detection from exhaust air filters and fecal pellet samples (Lupini et al., 2022).

Classical bacterial-culture-based microbiology remains a key challenge in routine health monitoring of laboratory rodents and is the focus of the present article. This work serves as a practical atlas for pathogen identification in rodent animal facilities, outlining standardized approaches for microbial diagnosis. The protocols and guidelines presented here can be readily implemented by laboratory animal facility personnel to support efficient pathogen detection and to facilitate the identification and elimination of unexpected or undesired infectious agents from animal colonies.

### Critical Parameters

#### *Basic Protocol 1*

Proper sample collection is strongly recommended, as correct sampling, including avoidance of overloading and ensuring sufficient material, is critical for successful bacterial identification using classical microbiology methods. Culture plates should be incubated overnight at 37°C in a $CO_2$-controled incubator. In addition, adequate quantities of fecal pellets are crucial for reliable DNA stool extraction PCR–based identification of *Helicobacter* spp.

#### *Basic Protocol 2 and Support Protocol 1*

Isolation of bacterial colonies from agar plates (blood, MacConkey, and others) for the purpose of differential testing should be performed with care to ensure that only a single colony is picked, as mixed colony sampling may compromise the accurate interpretation of the results.

### Troubleshooting

A list of common problems, along with their likely causes and solutions, is provided in Table 5.

### Understanding Results

Sample collection from mice and rats (Basic Protocol 1) constitutes the starting point of the pathogen identification workflow presented in Figure 1 and Table 1. Figure 2 outlines the sample processing steps on the first day, including inoculation onto agar plates and growth media (Basic Protocol 2). Table 2 presents the list of pathogens recommended by FELASA, together with additional bacterial species that may be isolated but are not included in the FELASA-recommended panels presented in Table 4.

The most critical part of this set of protocols is the accurate identification of bacterial colonies, their careful isolation, and their subsequent differentiation using selective and differential plates and media (Support Protocol 1), which together form the basis for the animal facility health monitoring report. Figure 3 illustrates the identification workflow for Gram-positive bacteria, and Figure 4 and Table 3 depict the identification of Gram-negative bacteria.

*Pseudomonas aeruginosa* identification is detailed in Figures 4 and 5, where a simple observation under UV light allows reliable identification.

*Helicobacter* spp., which are included in the FELASA-recommended pathogen panel, are identified using PCR analysis described in Basic Protocol 3 and Figure 6.

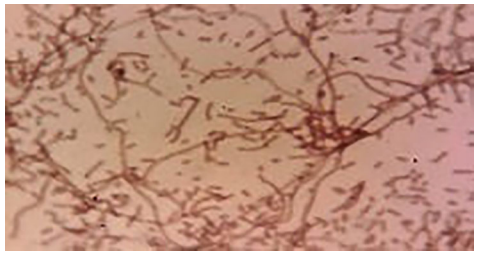
Parasitological screening represents a core component of routine health monitoring in mice and rats and is described in Basic Protocols 4 and 5. Direct microscopic examination for pinworms, mites, and eggs remains the most commonly used method in laboratory animal facilities. Figures 7 and 8 illustrate nematodes such as *Syphacia obvelata* and *Aspiculuris tetraptera*, highlighting key morphological characteristics of adult worms and eggs (Abdel-Gaber, 2016; Ain-Fatin et al., 2022). Figure depicts fur-skin ectoparasites and their eggs, identified based on morphological features (Whary et al., 2015). When parasites are

**Table 4** Bacterial Profiles Identified During Pathogen Screening That Are Not Included in the FELASA-Recommended Panels

| Bacteria | Colony appearance (blood agar) | MacConkey | Chromoagar orientation | Enterotest, citrate, urea | Gram staining | Selenite | Image |
|---|---|---|---|---|---|---|---|
| *Morganella morganii* | White hemolytic | Colorless hemolytic, with odor | Clear transparent, slight brownish background, lack of swarming which differs from that of *Proteus* spp. | Generally i (+) but sometimes i (-), c (-), u (+), Glu orange (-), ONPG cherry (-), motility clear | Gram-negative, long bacillus; previous name *Proteus morganii* (family Proteus) | Negative | |
| *Enterobacter cloacae* | Big, gray, spread, no limited borders, mucoid | Pink, sometimes colorless, different from *E. coli*, pink halo, sometimes elliptical | Metallic blue diffuse, mucoid pink halo | i (-), c (+), u (-), Glu yellow (+), ONPG orange (+), motility (+), dark slant, c (+) blue, u (-) yellow | Gram-negative, nicely–shaped bacillus, rod-shaped, micro-flagella | – | |

(*Continued*)

**Table 4** Bacterial Profiles Identified During Pathogen Screening That Are Not Included in the FELASA-Recommended Panels, *continued*

| Bacteria | Colony appearance (blood agar) | MacConkey | Chromoagar orientation | Enterotest, citrate, urea | Gram staining | Selenite | Image |
|---|---|---|---|---|---|---|---|
| *Yersenia enterolytica* | Big, white, round transparent halo | – | Blue | i (-), c (-), u (+), Glu orange (-), ONPG cherry (-) | Gram-negative bacillus | – | |
| *Enterococcus* spp. | Big, green | – | Diffuse with pink halo | i (-), c (-), u (+), Glu yellow (+), ONPG orange (+), gas (±), motility (-) | – | – | |
| *Citrobacter diversus* | – | – | Light blue with diffuse edges | i (+), c (+), u (-), Glu orange (-), ONPG orange (+), motility (+) | Gram-negative bacillus | – | |
| *Citrobacter rodentium* | – | – | – | i (±), c (+), u (-), Glu yellow (+), ONPG (-), no color change, $H_2S$ (+), gas (+), motility (+) | – | – | |
| *E. coli "Shigella*-like" strain G | Greenish, bad odor, *E. coli* lactose- | Colorless, mucoid | Colorless, slightly rose | i (+), c (-), u (-), Glu yellow (+), ONPG orange (+), gas (+) | Gram-negative, fat, bacillus-like | – |  |
| *Actinobacillus muris* | Beige, transparent, Pasteurella family | No grow | Blue, like enterococcus | i (-), c (-), u (-), Glu orange (-), ONPG orange (-), no reaction | Gram-negative, long bacillus, rod shaped | – | |

Abbreviations: i, indole (a colorless cap indicates a negative reaction, a pink cap indicates a positive reaction); c, citrate (green indicates a negative reaction, blue indicates a positive reaction); u, urea (yellow indicates a negative reaction, pink/rose indicates a positive reaction); Glu, glucose (orange indicates a negative reaction, yellow indicates a positive reaction); ONPG, *o*-nitrophenyl-β-galactoside (cherry indicates a negative reaction, orange indicates a positive reaction; gas, presence of bubbles in the medium due to $CO_2/H_2$ production from carbohydrate fermentation; (+) positive reaction; (-), negative reaction.

**Table 5** Troubleshooting Guide for Pathogen Identification in Soiled-Bedding Sentinel Animals

| Problem | Possible cause | Solution |
|---|---|---|
| Undefined bacteria | Bacterial colony was not properly isolated | Use a MALDI-TOF technique (Topić Popović et al., 2023). |
| Unclear result from the indole test | Mixed isolated colonies | Pick another colony from the original plate and repeat the test. |
| Gram staining showed both gram-positive and gram-negative bacteria | Mixed isolated colonies | Repeat the gram staining test, picking a colony from the original plate. |
| Overloaded inoculation plates | Too much cultured material | Re-collect samples, or dilute sample and re-plate. |
| No DNA detected in fecal samples | Not enough stool for DNA extraction using the Qiagen kit | The microcentrifuge tube should be half full (∼6-8 pellets of mouse feces or half a pellet of rat feces). |
| PCR *Helicobacter* primers contaminated | Tips or water contaminated the PCR primers | Dilute primers with new RNase-free water using filter tips. |
| No parasites (pinworms, mites or eggs) found when observed by microscope | Low load of positive parasites or beginning of the life cycle | Analyze fecal pellets combined with environmental cage swabs by PCR to confirm the results as PCR is more sensitive for parasite detection. |

detected, appropriate veterinary treatment protocols should be implemented to eradicate the parasites.

Regarding other pathogens, in most cases there is no effective therapeutic intervention that completely eradicates the infectious agent. Therefore, the most reliable method of control is depopulation of the affected colony followed by re-derivation and re-establishment of the colony under controlled conditions or from new breeding pairs.

Alternatively, if it is determined that the agent detected does not significantly interfere with research objectives, the rooms may remain colonized (e.g., in SPF facilities with agents such as MNV, *Helicobacter* spp., or *Pasteurella* spp.). In the case of certain viral infections, such as murine hepatitis virus (MHV), if detected in a room targeted for eradication, temporary cessation of breeding for ∼2 months may be sufficient to eliminate the infection (Artwohl et al., 2008).

For bacterial infections, antimicrobial therapy generally reduces pathogen burden but does not reliably achieve complete eradication.

Detection of viral pathogens described in Basic Protocol 6 and Table 2 is performed on serum samples by Charles River Research Animal Diagnostic Services.

## Time Considerations

Completion of the full microbiological analysis, including bacterial and parasite identification after sample collection (Basic Protocols 1, 2, 4 and 5), typically requires 1 week.

PCR testing for *Helicobacter* spp. (Basic Protocol 3) is completed within ∼3 days.

Confirmation and species-level identification of parasites require PCR-based analyses performed by Charles River Research Animal Diagnostic Services (Support Protocol 2). Virus identification based on serum analysis (Basic Protocol 6) is also performed by Charles River Research Animal Diagnostic Services. The total turnaround time, including sample shipment and receipt of results, may be up to 12 days.

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## Author Contributions

**Debora Rapaport**: Conceptualization; data curation; investigation; methodology; visualization; writing—original draft; writing—

review and editing. **Maya Levin Arama**: Data curation; writing—review and editing. **Michael Harlev**: Funding acquisition; supervision; writing—review and editing.

## Conflict of Interest

None.

## Data Availability Statement

All data that support the findings of this study are included within the article.

## Research Ethics

The present study for sentinel animal health evaluation at Tel Aviv University Animal Facilities was approved by the university Institutional Animal Care and Use Committee (IACUC), permit number TAU-MD-IL-2307-150-2.UAF.

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