Warning Signs:
A Practical Guide to Clinical Microbiology for the Infection Preventionist

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Objectives

• To review categories of clinically-relevant microorganisms and their common reservoirs.

• To emphasize important points to consider when interpreting a microbiology report.

• To highlight microbiological characteristics that should trigger an epidemiological or infection control response.
Clinical Microbiology Overview

- Bacteria
- Viruses
- Fungi
- Parasites
- Prions*

CDC, Public Health Image Library (PHIL)
Bacteriology
Bacterial Identification Techniques: Culture

- Growth media are broths (liquid) or agar (solid or semisolid) that provide the appropriate nutrients for a microbe to grow.
- Optimal incubation parameters are required for some organisms to grow (i.e. fastidious):
  - Temperature
  - Nutrients
  - Environment (oxygen, carbon dioxide, etc.)
  - Time
Step 1 & 2: Crystal Violet and Iodine enters bacterial cells, staining them purple
Purple = gram positive
Pink = gram negative

Step 3: Alcohol wash rinses stain away from cells with low peptidoglycan content (destain)

Step 4: Safranin counter stain turns cells decolorized in Step 2 pink
Gram Stain Morphology: Shape

- Rods
- Cocci
- Coccobacillus
- Curved Rod
- Fusiform bacillus

- Yeast
- Hyphae or Pseudohyphae

Murray et al., 7ed Medical Microbiology
Gram Stain Morphology: Shape & Arrangement of GPCs

- Chain
- Pair
- Tetrad
- Cluster
GPC in pairs with capsule

**Streptococcus pneumoniae** – cocci in capsule
Chains, or ‘end-to-end’

Spore-forming

Gram Stain
Morphology: Shape & Arrangement of Gram Positive Rods (GPRs)

Coryneform
Gram Stain Morphology:
Shape and Arrangement of Gram Negative Cocci and Coccobacilli

Diplococcus

Cocci

Coccobacillus

Pair = ‘diplococci’
GNDC
Gram Stain Morphology: Shape & Arrangement of Gram Negative Rods (GNRs)

- Long, Thin GNR (ex. Pseudomonas, Stenotrophomonas)
- Fusiform (ex. Fusobacterium, Capnocytophaga)
- Bipolar Staining (ex. Enterobacteriaceae)
Curved Gram Negative Rods
Gram Stain Morphology: Shape & Arrangement of Curved Gram Negative Rods

- **Curved rods**

- **ex. Campylobacter**

- **ex. Vibrio**
Bacteria of Greatest Concern Epidemiologically

**GPCs**
- S. aureus
- S. pneumoniae
- Group A Strep
- Group B Strep

**GNRs**
- Acinetobacter sp.
- Bordetella pertussis
- Brucella sp.
- Campylobacter sp.
- E. Coli O157
- Enterobacteriaceae*
- STEC
- Francisella tularensis
- Haemophilus influenzae (invasive)
- Legionella sp.
- Pseudomonas aeruginosa
- Salmonella sp.
- Shigella sp.
- Vibrio
- Yersinia sp.

**GPRs**
- Bacillus anthracis
- Clostridium botulinum
- Clostridium difficile
- Clostridium tetani
- Cornebacterium diptheriae
- Listeria monocytogenes
- Mycobacterium tuberculosis

**Gram Negative Cocci**
- Neisseria meningitidis

**Non-Culturable**
- Coxiella burnetii
- Bartonella sp.
### Important Clinical Bacteria by Gram Stain

<table>
<thead>
<tr>
<th>GPCs</th>
<th>GNRs</th>
<th>GPRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphlococcus</td>
<td>E. coli</td>
<td>Bacillus</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Klebsiella</td>
<td>Clostridium</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>Serratia</td>
<td>Lactobacillus</td>
</tr>
<tr>
<td>Abiotrophia</td>
<td>Enterobacter</td>
<td>Corynebacterium</td>
</tr>
<tr>
<td>Granulicatella</td>
<td>Citrobacter</td>
<td>Listeria</td>
</tr>
<tr>
<td>Micrococcus</td>
<td>Salmonella</td>
<td>Proprionibacterium</td>
</tr>
<tr>
<td>Rothia</td>
<td>Shigella</td>
<td>Mycobacterium*</td>
</tr>
<tr>
<td>Leuconostoc</td>
<td>Pseudomonas</td>
<td>Nocardia*</td>
</tr>
<tr>
<td>Aerococcus</td>
<td>Acinetobacter*</td>
<td>Erisepelothrix</td>
</tr>
<tr>
<td></td>
<td>Haemophilus*</td>
<td>Arcanobacterium</td>
</tr>
<tr>
<td></td>
<td>Capnocytophaga</td>
<td>Cardiobacterium</td>
</tr>
<tr>
<td>Gram Negative Cocci</td>
<td></td>
<td>Actinomyces</td>
</tr>
<tr>
<td>Moraxella</td>
<td>Kingella</td>
<td></td>
</tr>
<tr>
<td>Neisseria</td>
<td>Legionella</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter*</td>
<td>Eikenella</td>
<td></td>
</tr>
<tr>
<td>Haemophilus*</td>
<td>Yersinia</td>
<td></td>
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</tbody>
</table>
Virology
Viral Diagnostics

<table>
<thead>
<tr>
<th>PCR</th>
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<tbody>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>40</td>
</tr>
<tr>
<td>1,000,000</td>
</tr>
</tbody>
</table>

Cycles

Viral Culture

cdc.gov; ncbi.nlm.nih.gov

Uninfected

Infected
# Viruses by Primary Source of Isolation

<table>
<thead>
<tr>
<th>Diarrhea</th>
<th>Respiratory</th>
<th>Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrovirus</td>
<td>Rhinovirus</td>
<td>Cytomegalovirus (CMV)</td>
</tr>
<tr>
<td>Norovirus</td>
<td>Coronavirus</td>
<td>HSV 6</td>
</tr>
<tr>
<td>Sapovirus</td>
<td>Parainfluenza (I-IV)</td>
<td>HSV 8</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Respiratory syncytial virus (RSV)</td>
<td>JC Virus</td>
</tr>
<tr>
<td></td>
<td>Influenza (A/B)</td>
<td>BK Virus</td>
</tr>
<tr>
<td></td>
<td>Adenovirus *</td>
<td>Epstein-Barr Virus (EBV)*</td>
</tr>
<tr>
<td></td>
<td>Metapneumonvirus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enteroviruses*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MERS-CoV</td>
<td></td>
</tr>
</tbody>
</table>

## Vaccine-Preventable
- Measles
- Mumps
- Rubella
- Poliovirus
- VZV
- Hepatitis A
- Hepatitis B

## Chronic
- HIV
- HTLV
- Hepatitis C
- Hepatitis Delta Virus
- Human Papillomavirus (HPV)
- Herpes Simplex Virus 1/2
Zoonotic/Arboviruses

- Rabies
- Hantavirus
- Lymphocytic choriomeningitis virus (LCMV)
- Herpes B virus
- Yellow fever
- Encephalitis Viruses (many)
- Chikungunya
- Dengue
- Many others

Viruses of Immediate Concern

- Vaccinia virus (smallpox vaccine)
- Variola virus (smallpox)
- Monkeypox
- SARS
- Avian Influenza (H5 or H7)
- Ebola virus
- Crimean-Congo
- Eastern Equine Encephalitis (EEE)
- Lassa fever virus
- Lujo virus
- Marburg Virus
- 1918 Influenza Virus
- S. American Hemorrhagic Fever viruses
- Kyasanur Forest disease virus
- Omsk hemorrhagic fever virus
- Hendra virus
- Nipah virus
- Rift Valley fever virus
- Venezuelan equine encephalitis virus
Fungi
Fungal Diagnostic Techniques

Fungal Culture

Microscopic Evaluation
Is it…

Budding yeast??

OR

Hyphae??
Budding Yeast

With Pseudohyphae:

*Candida albicans*
*C. tropicalis*
*Trichosporon*

Without Pseudohyphae:

*Candida glabrata*
*Cryptococcus*
*Malassezia*

Or

Dimorphic Fungi
Hyphae

With Septa:

*Aspergillus*
*Fusarium*
*Alternaria*
*Exserohilium*

(Hyaline Molds, Dematiacious Molds, Dimorphic Fungi)

Without Septa:

*Mucor*
*Rhizopus*
*Rhizomucor*
*Apophysomyces*

(Mucorales)
# Fungal Overview

## Mucorales
- Rhizopus
- Mucor
- Rhizomucor
- Apophysomyces

## Hyaline Molds
- Aspergillus
- Penicillium
- Fusarium
- Scedosporium
- Many Others

## Dimorphic Fungi
- Sporothrix
- Histoplasma
- Blastomyces
- Coccidioides
- Paracoccidioides
- Penicillium marneffei

## Yeasts
- Candida
- Cryptococcus
- Trichosporon
- Malassezia

## Dermatophytes
- Trichophyton
- Microsporum
- Epidermophyton

## Dematiaceous Molds
- Alternaria
- Exserohilium
- Others

## Other
- Pneumocystis
Parasitology
Parasitology Techniques

Stool Culture

Microscopy

Entamoeba histolytica, Trichrome stain
Parasitology Overview

- **Blood**
  - Plasmodium
  - Babesia
  - Leishmania
  - Trypanosoma
  - Microfilaria

- **Muscle**
  - Trichinella

- **Urogenital**
  - Trichomonas

- **Bone Marrow**
  - Lesimania*
  - Plasmodium*
  - Trypanosoma*

- **Liver/Spleen**
  - Echinococcus

- **Brain/CSF**
  - Taenia solium
  - Echinococcus
  - Naegleria
  - Acanthamoeba
  - Balamuthia
  - Toxoplasma
  - Microsporidia

- **Lungs**
  - Paragonimus
<table>
<thead>
<tr>
<th>Stool Parasitology Overview</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entamoeba histolytica</td>
</tr>
<tr>
<td>Blastocystis hominis</td>
</tr>
<tr>
<td>Giargia lamblia</td>
</tr>
<tr>
<td>Dientamoeba fragilis</td>
</tr>
<tr>
<td>Cryptosporidum</td>
</tr>
<tr>
<td>Cyclospora cayetanensis</td>
</tr>
<tr>
<td>Cystoisospora belli</td>
</tr>
<tr>
<td>Enterobius vermicularis</td>
</tr>
<tr>
<td>Hookworm</td>
</tr>
<tr>
<td>Strongyloides</td>
</tr>
<tr>
<td>Taenia</td>
</tr>
<tr>
<td>Trichuris tricuria</td>
</tr>
<tr>
<td>Sarcocystis</td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
</tr>
<tr>
<td>Diphyllobothrium latum</td>
</tr>
<tr>
<td>Clonorchis sinensis</td>
</tr>
<tr>
<td>Paragonimus</td>
</tr>
<tr>
<td>Schistosoma</td>
</tr>
<tr>
<td>Fasciolopsis</td>
</tr>
<tr>
<td>Enterobius*</td>
</tr>
</tbody>
</table>
Prions

phil.cdc.gov
- Transmissible spongiform encephalopathies
- Rare, progressive neurodegenerative disorders that affect both humans and animals
- Prion disease is caused by the accumulation of misfolded proteins

<table>
<thead>
<tr>
<th>Human Prion Diseases:</th>
<th>Animal Prion Diseases:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creutzfeld-Jakob Disease (CJD)</td>
<td>Bovine Spongiform Encephalopathy (BSE)</td>
</tr>
<tr>
<td>Variant CJD (vCJD)</td>
<td>Chronic Wasting Disease (CWD)</td>
</tr>
<tr>
<td>Fatal Familial Insomnia</td>
<td>Scrapie</td>
</tr>
<tr>
<td>Kuru</td>
<td></td>
</tr>
</tbody>
</table>
Important points to consider when interpreting a microbiology report.
A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)
Microbiology Pearl #1

A test is only as good as the specimen submitted.
For Labs that perform direct Gram stains:  
“The laboratory has protocols in place to use Gram stain results to provide a preliminary identification of organisms, evaluate specimen quality when appropriate, and to guide work-up of cultures.”
Respiratory Specimens

- Assessment of specimen quality using low-powered magnification

- Generally this is based on the ratio of PMNs (neutrophils) to epithelial cells

Read: >10 SEC/LPF or “Many squamous epithelial cells”

http://clinmicro.asm.org
Respiratory Specimens

- If a respiratory specimen is considered “good quality”
  - Generally up to 3 “potential pathogens” are worked up.
  - Workup may be guided by the gram stain result.

- Respiratory gram stains report only predominant morphologies above the background of normal oral flora.
  - Moderate mixed flora
  - Many Gram positive diplococci.
  - Few Gram negative rods.
  - Many Gram positive cocci in clusters.

http://clinmicro.asm.org
Microbiology Pearl #2

The Laboratory report will not list everything that grows. Specimen source dictates workup.
Microbiology Standard

With the exception of a quantitative culture (ex. Urine) labs that perform culture will report a qualitative assessment of the number of colonies growing for a particular organism.

Example

- **1 COLONY**
- **SCANT** [2 colonies]
- **LIGHT** [3-10 colonies]
- **MODERATE** [>10 colonies in the 1\textsuperscript{st} and 2\textsuperscript{nd} streak quadrants]
- **HEAVY** [>10 colonies into the 3\textsuperscript{rd} and 4\textsuperscript{th} streak quadrants]
Example #1: Physician requests gram stain and culture on a patient suspected of having bacterial pneumonia.

- **Gram Stain:** Many neutrophils, many gram negative rods.
- **Culture grows:** moderate *Pseudomonas aeruginosa*, moderate *E. coli*, moderate *Proteus* sp., few Diptheroids
- **Workup:** *P. aeruginosa*, *E. coli*, *Proteus* sp.
Example #2: Physician requests gram stain and culture on a patient suspected of having bacterial pneumonia.

- **Gram Stain:** Many neutrophils, few epithelial cells, many GPCs in clusters, few GNRs, many mixed oral flora.
- **Culture grows:** moderate coagulase-negative *Staphylococcus*, moderate Diptheroids, few *E. coli*, rare *Staphylococcus aureus*
- **Workup:** *E. coli*, *S. aureus*
Case:

A central venous catheter tip is received in the microbiology laboratory for culture. It is plated to blood agar plate using the roll plate method. The catheter tip culture is growing a total of 4 colonies.

Two sets of blood cultures collected within 24 hours of the catheter tip culture were received in the laboratory; these are negative.

How should the lab proceed with work up of the catheter tip culture?

A. Identify the organism and perform susceptibility testing.
B. Perform susceptibility testing if the organism identified is *Staphylococcus hominis*.
C. Perform susceptibility testing if the organism identified is *Candida albicans*.
D. Report that the catheter tip culture is clinically insignificant.
Microbiology Pearl #3

Be specific about the body site and type of wounds or fluids.
In the case of wounds, the specimen of choice is a biopsy of the advancing margin of the legion. A swab of a wound is not as optimal for diagnostic purposes.

- Burns
- Human Bites
- Animal Bites
- Trauma-Associated
- Surgical Site
- Interventional Radiology/Drain Device Associated
Microbiology Pearl #4

“A strong, ongoing partnership between microbiology laboratory professionals and infection preventionists should remain a top priority in all infection prevention programs to ensure maximum patient safety and positive patient outcomes.”

- The Infection Preventionist’s Guide to the Lab
Bacterial Resistance

**GNRs**
- MDR *Acinetobacter baumanii* complex
- *Enterobacteriaceae* *
  1.) ESBL
  2.) CRE
  3.) CP-CRE: KPC, NDM-1
- MDR *Pseudomonas aeruginosa*

**Staphylococcus aureus**
- MRSA
- ViSA
- VRSA

**Enterococcus faecium or Enterococcus faecalis**
- VRE

**GPRs**
- *Mycobacterium tuberculosis* (MDR, XDR)
Appendix A. Suggestions for Confirmation of Resistant (R), Intermediate (I), or Nonsusceptible (NS) Antimicrobial Susceptibility Test Results and Organism Identification

<table>
<thead>
<tr>
<th>Organism or Organism Group</th>
<th>Resistance Phenotype Detected(^a)</th>
<th>Category I</th>
<th>Category II</th>
<th>Category III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not reported or only rarely reported to date</td>
<td>Uncommon in most institutions</td>
<td>May be common, but is generally considered of epidemiological concern</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Action Steps: (^a)</td>
<td>Confirm ID and susceptibility</td>
<td>Confirm ID and susceptibility if uncommon in your institution</td>
<td>Confirm ID and susceptibility if uncommon in your institution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report to infection control.</td>
<td>Check with infection control in your facility to determine if special reporting procedures or further action are needed.</td>
<td>Check with infection control in your facility to determine if special reporting procedures or further action are needed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Send to public health laboratory.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Save isolate.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NOTE: May be appropriate to notify infection control of preliminary findings before confirmation of results.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism or Organism Group</th>
<th>Resistance Phenotype Detected(^a)</th>
<th>Category I</th>
<th>Category II</th>
<th>Category III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any Enterobacteriaceae</td>
<td>Carbapenem – I or R (^b)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amikacin, gentamicin, and tobramycin – R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli Klebsiella spp. Proteus mirabilis</td>
<td>Extended-spectrum cephalosporin (^b) – I or R</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Salmonella and Shigella spp.</td>
<td>Cephalosporin III – I or R</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluoroquinolone – I or R</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>Colistin/polymyxin – R</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carbapenem – I or R</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Colistin/polymyxin – I or R</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Amikacin, gentamicin, and tobramycin – R</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Carbapenem – I or R</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SELECT AGENTS AND TOXINS LIST

The following biological agents and toxins have been determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products. An attenuated strain of a select agent or an inactive form of a select toxin may be excluded from the requirements of the Select Agent Regulations. Here is a list of excluded agents and toxins.

HHS and USDA Select Agents and Toxins
7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73
Classify the Microorganisms by Risk Group and Biosafety Level

A. HSV  
B. E. coli K12  
C. Candida albicans  
D. Ebola virus  
E. Brucella abortus  
F. Salmonella  
G. Mycobacterium tuberculosis  
H. Marburg virus  
I. Coccidioides immitis

BSL-1  
BSL-2  
BSL-3  
BSL-4
Answers

• **BSL-1:**
  – *E. coli* K12

• **BSL-2:**
  – *Candida albicans*
  – HSV
  – *Salmonella*

• **BSL-3:**
  – Brucella abortus
  – *Mycoplasma tuberculosis*
  – Coccidioides immitis

• **BSL-4:**
  – Marburg Virus
  – Ebola Virus
IN CASE OF EMERGENCY

Call THE MICRO LAB
When to always call the laboratory…
… Gram Stains

<table>
<thead>
<tr>
<th>Specimen …</th>
<th>You Read ....</th>
<th>Your concern …</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>GNDCs</td>
<td><em>Neisseria meningitidis</em></td>
</tr>
<tr>
<td></td>
<td>GPRs</td>
<td><em>Bacillus anthracis</em></td>
</tr>
<tr>
<td></td>
<td><em>Listeria</em></td>
<td></td>
</tr>
<tr>
<td>GNRs</td>
<td><em>Haemophilus influenzae</em></td>
<td></td>
</tr>
<tr>
<td>Blood Culture</td>
<td>GPR with spores</td>
<td><em>Bacillus anthracis</em> (and others)</td>
</tr>
</tbody>
</table>
## Culture Results

<table>
<thead>
<tr>
<th>Source</th>
<th>You Read …</th>
<th>The Concern…</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANY</td>
<td>Bacillus anthracis</td>
<td>Anthrax</td>
</tr>
<tr>
<td></td>
<td>Yersinia pestis</td>
<td>The Plague</td>
</tr>
<tr>
<td></td>
<td>Burkholderia pseudomallei</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Burkholderia mallei</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Franciscella tularensis</td>
<td>Rabbit Fever</td>
</tr>
<tr>
<td></td>
<td>Brucella sp.</td>
<td>Brucellosis</td>
</tr>
<tr>
<td></td>
<td>Corynebacterium diptheriae</td>
<td>Dipheria</td>
</tr>
<tr>
<td></td>
<td>Listeria</td>
<td></td>
</tr>
<tr>
<td>Wound</td>
<td>Clostridium perfringens</td>
<td></td>
</tr>
<tr>
<td>Sputum</td>
<td>Mycobacterium tuberculosis</td>
<td>TB</td>
</tr>
<tr>
<td>Vaginal</td>
<td>β-hemolytic Strep</td>
<td>Group B Strep (pregnancy)</td>
</tr>
</tbody>
</table>

...
## Ordered Tests

<table>
<thead>
<tr>
<th>Source</th>
<th>You Read …</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANY</td>
<td>Rule out for Agent of Bioterrorism</td>
</tr>
<tr>
<td></td>
<td>Parvovirus B19</td>
</tr>
<tr>
<td></td>
<td>VZV PCR or Serology</td>
</tr>
<tr>
<td></td>
<td>Measles PCR or Culture</td>
</tr>
</tbody>
</table>
What is the Role of the Microbiology Laboratory?

1. Clarify the presence of infection. Clinical presentation *must* guide the microbiological workup and all results *must* be interpreted clinically!

2. Specify the etiology of infection.

3. Motivate the appropriate selection of antimicrobial agents

4. Assist in identification, control, and prevention of nosocomial or public health infections
WHEN IN DOUBT

Call THE MICRO LAB