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TEXTBOOK

MEDICAL MICROBIOLOGY, VIROLOGY, IMMUNOLOGY

Editors V.V. Zverev, M.N. Boichenko

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LIST OF ABBREVIATIONS

♠ — trade name of medication
℘ — medication not registered in the Russian Federation
ACA — anticomplementary activity
ADA — adenosine diphosphoric acid
AIDS — acquired immunodeficiency syndrome
ALA — antilysozyme activity
ATP — adenosinetriphosphoric acid
BCG — Bacillus Calmette-Guerin
Ca^{+} — calcium ions
cAMP — cyclic adenosine monophosphate
CCHF — Crimean-Congo Hemorrhagic Fever
CF — colonization factors
CFT — complement-fixation test
CJD — Creutzfeldt-Jakob disease
Cl^{-} — chloride ions
DL — lethal dose
DNA — desoxyribonucleic acid
DTH — delayed type hypersensitivity
EAEC — enteroadherent Escherichia coli
EB — elementary bodies
EBV — Epstein–Barr virus
EEE — Eastern equine encephalitis
EHEC — enterohemorrhagic Escherichia coli
EIA — enzyme-immunoassay
EIEC — enteroivnasive Escherichia coli
EPEC — enteropathogenic Escherichia coli
ETEC — enterotoxigenic Escherichia coli
FHA — filamentous hemagglutinin
GIT — gastrointestinal tract
HAI — hemagglutination inhibition test
HBV — hepatitis B virus
HCV — hepatitis C virus
HDV — hepatitis D virus
HFRS — hemorrhagic fever with renal syndrome
HGA — human granulocytic anaplasmosis
HHV — human herpesviruses
HIV — human immunodeficiency virus

HME — human monocytic ehrlichiosis
HPIVs — human parainfluenza viruses
HPV — human papilloma virus
HSP — heat shock protein
HSV — herpes simplex virus
IFA — immunofluorescence assay
Ig — immunoglobulins
IHA — indirect hemagglutination assay
IIFT — indirect immunofluorescence test
IL — interleukin
LPS — lipopolysaccharide
LT — heat-labile enterotoxin
MOMP — major outer membrane porin
Na^+ — sodium ions
NAD — nicotinamide adenine dinucleotide
NT — neutralization test
NTM — nontuberculous mycobacteria
PA — precipitation assay
PCR — polymerase chain reaction
PHA — passive hemagglutination assay
RB — reticulate bodies
RIA — radioimmunoassay
RIHA — reverse indirect hemagglutination assay
RNA — ribonucleic acid
RSV — respiratory syncytial virus
SF — spotted fever
T3SS — type 3 secretion system
T3SSP — type 3 secretion system proteins
T4SS — type 4 secretion system
TBEV — tick-borne encephalitis virus
TBSF — tick-borne spotted fever
VEE — Venezuelan equine encephalitis
WEE — Western equine encephalitis
WHO — World Health Organization

Part III

SPECIAL MICROBIOLOGY

Chapter 14
MICROBIOLOGICAL AND IMMUNOLOGICAL DIAGNOSIS

14.1. CHARACTERISTICS OF MICROBIOLOGICAL, VIROLOGICAL AND IMMUNOLOGICAL LABORATORIES

All work with microbes is carried out in laboratories, which, depending on their primary objectives, can be of research nature, diagnostic or manufacturing.

The health care system includes:

- general and special (biochemical, bacteriological, immunological, cytological, etc.) clinical diagnostic laboratories, which are parts of hospitals, outpatient clinics, dispensaries and other healthcare facilities;
- bacteriological laboratories of the State Committee for Sanitary and Epidemiological Oversight (Gossanepidnadzor, GSN);
- sanitary and bacteriological laboratories of the GSN;
- sanitary and chemical laboratories of the GSN;
- central (central research laboratory — CRL), task-specific, industry-specific, and university laboratories;
- specialized laboratories (for highly infectious diseases, etc.).

Nowadays laboratories and larger laboratory facilities (departments, institutes, manufacturing plants) are usually task-oriented and work with a certain group of microorganisms.

Virology laboratories allow for working with viruses, as they have appropriate equipment at their disposal, and use special research methods. There are mycological and protozoological laboratories. Bacteriological laboratories are also getting specialized; they target certain groups of bacteria, such as rickettsia, tuberculosis, leptospiral, anaerobic, and other organisms. Immunological investigations are carried out in immunological laboratories. Specially equipped laboratories allow for working with pathogenic microorganisms. They ensure the operating mode and safety and exclude the possibility of staff contamination or microbial leakage outside the laboratory.

The necessity of well-managed regulation of the conditions of work with microorganisms with a variable degree of danger for the laboratory staff and the surrounding population, led to the development of a classification of microorganisms, dividing them into 4 groups according to their biological risk [the World Health Organization (WHO) classification]. In the Russian Federation, in accordance with the WHO recommendations, pathogenic microorganisms are also divided into 4 groups:

- group 1: pathogens of highly dangerous infections (plague, natural smallpox, yellow fever, Ebola);
- group 2: pathogens of highly contagious epidemic human diseases [anthrax, brucellosis, spotted fever (SF)];
- group 3: pathogens of infectious diseases, singled out into separate nosological groups (typhoid fever, shigellosis, diphtheria);
- group 4: opportunistic microorganisms that cause opportunistic infections.

The numbering of the groups of microorganisms adopted in the Russian Federation differs from the WHO classification by a reverse order, where the first group includes microorganisms with the lowest pathogenicity, and the fourth group — highly dangerous ones.

Laboratories are also classified according to the grouping of microorganisms based on the level of the biosafety. According to the WHO nomenclature, microbiological laboratories are classified into 3 categories:

- basic (standard or general) laboratories which may be equipped with various protective equipment connected to their specific operating modes;
- high-security (isolated) laboratories
- maximum security laboratories with the highest level of isolation.

The safety of work in the laboratories of all categories is ensured by:

- adherence to the regulations and rules for laboratory work;
- compliance with the requirements for laboratory premises and their equipment;
- provision of laboratories with appropriate equipment;
- medical monitoring of the health of staff members;
- staff education and training about safety rules in the laboratory.

14.2. EQUIPMENT OF MICROBIOLOGICAL AND IMMUNOLOGICAL LABORATORIES

The premises of the basic laboratory must be spacious enough to ensure safe performance of laboratory work. The walls, ceiling and floor must have a smooth, easily washable surface, liquid-proof, resistant to the disinfectants

commonly used in the laboratory. The surface of the workbenches must be water-proof, resistant to disinfectants, acids, alkali, organic solvents and moderate heating. The laboratory furniture must be solid. The space under the tables and between furniture items must be easily accessible for cleaning. The laboratory must be equipped with an autoclave for waste decontamination. Waste containers should be marked in yellow.

The equipment of the basic laboratory must restrict or prevent the contact of the microbiologist with the infectious material; it must be made of durable corrosion-resistant liquid-proof materials. The equipment must be designed and installed so that it can be easily cleaned, decontaminated and inspected.

The laboratory is to be equipped with a microscope, autoclave, heating blocks, drying and sterilization rooms, a coagulation apparatus, a distillation apparatus, centrifuges, laboratory scales, a *pH* meter, a color density meter, a magnetic bar stirrer, and a washing bath.

Laboratory premises must be supplied with hot and cold water, electricity, vacuum, oxygen, high pressure air, etc. Some rooms are equipped with boxes and fume hoods.

The mandatory premises include laboratories of intestinal, airborne infections, sanitary-bacteriological, serological, as well as auxiliary rooms: a media preparation system, washing room, a sterilization room (clean and dirty zones), reception desk, storage rooms, sanitary facilities for staff, and a vivarium. Laboratories with facilities for bacterial carriage examination are additionally equipped with a reception desk, a procedure room, and a toilet room for collection of the material.

The premises are arranged so that the flows of dirty and clean material do not cross or contact each other.

The facilities of high-security laboratories should meet the same requirements as the basic ones. In addition, the laboratory of this type must be separated from the parts of the building where the movement of staff is not restricted. Hand washing equipment must be fitted with a foot pedal valve or an elbow control tap for turning on the water. Windows must be closed and taped up. The entrance doors to the laboratory premises must be self-locking and key-locking. The exhaust ventilation is designed so that the lowest pressure is maintained in the premises with the highest risk of infection. In this case, the air flow goes from the auxiliary premises towards the main working space. The extracted air is released into the environment only after filtration through bacterial filters. High-security laboratories are equipped according to the recommendations developed for the basic laboratories, in addition that all work with infectious material in such laboratities is performed in protective boxes.

Laboratories of maximum security include a number of features ensuring the maximum biological safety of the staff, population and environment. The entry and exit from the laboratory is performed through a medical decontamination station. When entering, the putting on cleanroom garments is mandatory; when leaving, targeted sanitary treatment of staff (shower, disinfectants) is essential before changing the clothes. In order to reduce the risk of infectious material leaking into the environment, the work is performed in boxes. The tabletop or laminar ***boxes*** allow to form physical barriers preventing possible contacts of the staff with infectious material.

14.3. SAFETY RULES IN MICROBIOLOGICAL LABORATORY

Fundamental safety rules in basic laboratory are as follows:

- prohibition against pipetting with the help of mouth;
- prohibition against eating, drinking, smoking, keeping food and applying cosmetics in the working area;
- maintenance of cleanliness and order;
- disinfection of working surfaces at least once a day and after each contact with infectious material;
- washing of hands after working with infectious material, animals, and before leaving the laboratory;
- performance of all types of work in a manner to minimize the possibility of aerosol formation; and
- decontamination of all infected materials prior to their disposal or reuse.

14.4. METHODS OF MICROBIOLOGICAL DIAGNOSIS OF INFECTIOUS DISEASES

Microbiological diagnosis allows making or confirming the clinical diagnosis of an infectious disease, determining the source of infection and the sensitivity of the pathogen to antibacterial drugs.

In the microbiological laboratory, various methods of diagnostis are used:

- microscopy;
- culture;
- biological;
- serological;
- allergological;
- molecular genetic methods.

The ***microscopic method*** means detection of mocroorganisms in the test material and their primary morphological identification.

Micropreparations can be native (they study living microorganisms); at the same time mobility of the studied objects can be determined. Microscopy of such preparations is carried out in the dark field of a light microscope, as well as using dark-field and phase-contrast microscopy.

The study of fixed preparations and those stained with various methods allows determining the location of microorganisms in the preparation, their sizes, cell components, reaction to staining. There are different microscopic techniques: with the help of a light, luminescent, electron microscope, etc.

Light microscopy is practically not used in the study of virus-containing material, due to the infinitesimal size of viruses. With the help of a light microscope, it is possible to detect intracellular inclusions that form in the affected cells in some viral infections.

In this case, the electron microscope is most commonly used, less often — the luminescent one. The light microscope can be applied only for detection of large viruses, using methods of supercoloration.

The microscopic method is often used as an approximate one, since it is not always possible to identify a microorganism by morphological characteristics.

The **cultural method** consists in plating the test material on artificial culture media, chicken embryos or cell cultures in order to isolate and identify the pure culture of the pathogen. This method is the "golden standard" of microbiological research, because it allows successful isolation and identification the pathogen, although it is expensive, time-consuming and lengthy as it takes 3–5 days, sometimes more).

This method is referred to as:

- ***bacteriological*** when growing a bacterial culture that is identified according to its morphological, tinctorial, cultural, biochemical and antigenic properties (if necessary, it allows epidemiological labeling and determination of susceptibility to antibiotics);
- ***mycological*** during the cultivation of fungi (plating of the studied material is carried out on Sabouraud medium);
- ***protozoological*** (parasitological) when isolating a protozoan;
- ***virological*** when working with virus-containing material.

Isolation of a pure culture is carried out in several stages. Sometimes, before plating, primary microscopy of the test material is carried out (optional stage). Urine, blood, feces, swabs from the throat and nose are not subject to primary microscopy. Pus, cerebrospinal fluid, sputum (from dilutions 10^4–10^5) are subject to microscopy before plating, as they help to choose the medium for plating.