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ABSTRACT

The contemporary society, pressured by globalization, requires accurate and reliable but above all comparable results. An important tool for advering comparability is ensurance of result traceability. The goal of each diagnostic microbiological laboratory, whose work is based on methods of cultivation of bacteria, is to set up fast and reliable microbiological diagnosis. In bacteriology, cultivation and reproduction of MM is the foundation of direct proof of bacteria from samples so the accuracy in preparation of the MM is imperative. Testing laboratories determine control procedures of MM prepared from dehydrated base as well as the ways to control technological processes of preparation according to DIN EN ISO 11133:2014. To prepare MM dehydrated bases that are produced in accordance with the wording of the pharmacopoeia (EP, USP) and reference to international standards (ISO, APHA) are used. The physical parameters that are controlled by the MM are: the pH value, the thickness of the plates, filling volume, color, clarity, consistency. The microbiological parameters that are controlled include sterility and productivity and selectivity of the certified reference material (CRM). CRM contain microorganisms of stable characteristics that are typical for a particular microbial species, and is suitable for proving the effectiveness of a particular MM and was purchased from internationally acknowledged collections. Quality microbiological media is one of the most important factors in the work of microbiological laboratories for bacteriological diagnosis. A proper implementation of the technological process of preparing is one of the key requirements in the system of implementation of quality control of the MM. The ISO/IEC 17025:2007 international standard require from laboratories to evaluate the measurement uncertainties of their results. An excessive measurement uncertainty calls into question the possibility of making the right decision based on these results (it refers to making a precise diagnosis or estimating therapeutic efficacy).

Keywords: microbiological media, accreditation, healthcare quality assurance

The infection involves entering the pathogen in host tissues, their maintenance, propagation, release of harmful substances for the host, and the host's reaction to all this.

Over the past decade, thanks to continuous research and development of laboratory techniques different bacterial species were discovered that cause infectious diseases as well as the source and routes of transmission of infections.

The disease occurs if all the elements in the Vograliko chain are present:

1. The source of infection may be everything that was in contact with the patient.
2. Way of spreading the infection: air, food, water, insects, touch..
3. Entrance door. Different microorganisms must enter the human body to cause disease.
4. The number of microorganisms. The more virulent the microorganism is, the smaller number of colonies for infection is needed.
5. The layout and exposure. Susceptibility, nutritional status, hygiene, age, sex, mental and physical fatigue are affected and it is important for the exposure how long the human body has been exposed to the microbes.

After the entry of a pathogenic microorganism an incubation period usually follows. At this time, which is different for different microorganisms, certain changes occur in the human body which are symptoms of disease.

Within the public health activities microbiological tests are covered [1] consisting of isolation and identification of microorganisms from:

- food
- water
- soil and air
- generally used items
- medical products
- clinical material in human medicine.

Free flow of goods and the globalization of markets have multiplied the share of diseases caused by food poisoning. Food safety requires the availability of laboratory data at the right time to all participants in the chain of production and supply. In this way the exchange and the flow of information are used in order to implement and ensure the food safety system. Today's way of producing, processing, storage, transport and sale sometimes adversely affects the quality, ie. the hygienic safety of food, and it plays an important role in the etiology of a variety of human diseases. That is why food security is very important, materialized part of nutrition and primary health care and security strategy can only be based on the principles of good hygiene and manufacturing practices (agricultural, veterinary, food technology), and on the basis of a critical assessment of acceptable risk and control of critical production points.

Microbiological hazards in food are a major source of foodborne illness in humans and can cause economic losses to food production and the food industry. To ensure the safety of food that reaches the consumer, laboratory tests are carried out on a daily basis whose results are guidelines for the further handling of food. The laboratory is one of the key elements in the chain of the food safety system and management system of food safety and risk management. Food safety management system is a very complex system through which hazards are detected and risks are assessed and it has become necessary to protect the health of consumers. Management of a system is daily involvement through monitoring of many parameters and records, as well as regular analysis of data in order to eliminate gaps and maximize the safety of consumers and establish functionality.

Availability of the results and rating them, enables quick and effective response in cases of harm to human health. European Food Safety Authority - EFSA lays down procedures in matters of food safety. By changing the approach in monitoring and ensuring, the health safety of the laboratories were given an indispensable role and value. The laboratory performs a critical part of the system of food safety and using the results of its activities determines the need for corrections at the time of production, and that the final products meet defined specifications, safety requirements and quality standards. Activities and organization of the laboratory over time have been changed according to the needs of the population and the demands of health care.

Laboratory preparation of the MM prepares different types of microbial surface that are used for the diagnosis of urinary tract infections, and tuberculosis, for diagnosing infection of the genital system, the diagnosis of infections of the respiratory system, skin and mucous membranes, for the diagnosis of infections of the gastrointestinal system, and the mycological and parasitological diagnosis [2]. MM are also being prepared to be used for testing the safety of food and general use, for the microbiological examination of the air, for monitoring, studying and assessing the health of drinking water, for testing the quality of wastewater, surface water and water for recreation, and sea water quality and monitoring of the water supply in the Split-Dalmatia County [2, 3, 4]

Over the past decade, thanks to continuous research and development of laboratory techniques, different bacterial species were discovered that cause infectious diseases as well as the sources and routes of transmission of infections.

Microbiological media are used for the cultivation of microorganisms in the laboratory. With their composition and characteristics they provide living conditions for microorganisms that are in the natural environment. This cultivation of microorganisms is referred to as in vitro cultivation, live cell in vivo. Microbiological media are different, complex chemical compounds, which microorganisms need to perform basic life functions: growth and reproduction. Different pathogens, strains of bacteria (bacteria cultivated in vitro) grow in different microbial surfaces. Therefore, MM are used for the

isolation, identification of microorganisms in microbiological tests (samples of human origin, food, water, air, soil).

Interpretation of results, based on analysis of environmental samples leads to the risk factors that affect the health of the population SDŽ. In this way, by performing microbiological activity that is of interest for the area of SDŽ, the quality of life of residents SDŽ is improved by reducing health risk factors: higher quality food, and improving the living and working environment through the preservation of the quality of drinking water.

A great number of MM is used for the cultivation of microorganisms. Good MM must have the following characteristics: selectivity, precision, ease of bacteriological analysis and speed detection.

Selective microbiological media have such composition and incubation conditions that prevent the growth of other microbial species other than those for which that MM is intended MM. The precision of microbiological media refers to determining more accurate number of microorganisms in the sample. Simple laboratory procedures allow processing of a large number of samples per unit time.

Speed in the detection of microorganisms is important in order to obtain findings rapidly from the time of delivery of the sample to the laboratory to the issuance of the final results. In this way it enables faster response to certain situations (incidents of environmental events, food poisoning, water pollution ...) that are dangerous to human health.

Bacteria are grown to be identified, studied and see to which antibiotics they are sensitive. We're doing that to produce vaccines and create useful mutants (those that produce human insulin or antibiotics).

In the process of the cultivation of microorganisms the specific type of nutrients and the incubation temperature and the atmospheric conditions (aerobic, microaerophilic or anaerobic) should be observed. The microorganisms may be cultured in a variety of containers (test tubes, petri dishes, different bottles).

The cultivation of microorganisms is possible in solid or in liquid microbiological media. Growing on solid has several advantages over the cultivation in liquid media. On the rigid substrate the number of bacteria colony forming units (eng. Colony Forming Units, CFU) in the sample can be determined.

Microbiological media are different:

According to the composition:

- simple substrates - not suitable for the cultivation of all bacteria. The simple ones contain only one essential ingredient (eg. meat broth, peptone water). They are used for the preparation of complex substrates.
- complex base - containing different substances which allow the growth of microorganisms of different qualities.

According consistency:

- The current surface - basically a broth or peptone water. Peptones are polypeptides which are obtained by proteolytic degradation of the meat. Extras: sugars, colors, indicators, serum, bile, blood.
- There is a blurring, cobweb formation, sometimes residue, skin on the surface, hemolysis (clears up), sometimes a gas bubble. Based on the increase in these substrates it is not possible to identify the microorganism. They are used for increased growth of bacteria.
- Solid media - containing 1.5% to 3.0% agar. Agar is a polysaccharide, is used for solidification of liquid culture media. The agar is prepared by extraction from seaweed *Gellidium corneum*, so that it is heated to 100 degrees and thus is dissolved, then dried and put into water to swell, and cooled to below 45 degrees to solidify and thus obtain a gelatinous mass containing agar pentose and agar pectose. This is the basis for the formation of rigid substrates and it is called the neutral agar. With the addition of 10% of the blood it is called blood agar, and from blood gain if chocolate is heated to hemoglobin in mata depths. For them it is possible to get a pure culture of bacteria.
- ➢The semi - containing 0.5% to 1.5% agar. Used to test the mobility of bacteria.

According to purpose:

- For the increased number of microorganisms: nutrient broth, blood agar, chocolate agar- Transport media: for transferring samples to the laboratory
- Differential background: used for the differentiation and classification of bacteria
- Selective media: blood agar, serum agar, protective agar, serum broth. These are enriched substrates, contain essential amino acids (from blood, serum, bile).
- At a time when requirements for more effective methods are constantly growing substrates that have the biochemical mechanisms are recommended by which at the suspect colonies can easily and clearly be distinguish. Reliability of detection and orientation of analysts on a background is very important. Therefore, it is necessary to thoroughly study the mechanism of substrate, as well as to collect practical information on the microorganisms that can grow on a given surface. The effect of recognizing the typical colony is the interaction surface, variability of microorganisms and analysts.

Proper and controlled prepares agar and liquid foundation from dehydrated base is a key factor in the optimal detection of microorganisms. At time when requirements for more effective methods are constantly growing standardized and controlled MM is a necessity. Quality foundation is to integrate all the

processes of preparing substrates in accordance with the ISO 17025:2007 standard. Production according to this process is standardized and thus establishing an internal control of the quality of produced substrates.

Supervision of accredited laboratories is performed by Croatian Accreditation Agency (CAA) supervising the maintenance of the management documents and the quality of the laboratory. In order to prove the conformity of the work process in the laboratories of the Member States of the European Union (EU) is established European infrastructure for quality control prices (certification and inspection bodies).

Their competence assessed and monitored by national accreditation bodies which are united in the European Organization for Accreditation (EA). Thus guaranteeing the quality of services and products, the safety and protection of the environment and human health in all Member States [5]. Thus, accreditation is a process by which the CAA supervises work in specified institutions and confirms that they are professionally and technically qualified to work in line with European standards in the Croatia adopted as national standards (DIN) [6]. Standard ISO/IEC 17025:2007 is a fundamental norm of the technical competence of laboratories [7].

By the introduction of the management system of quality control the procedures of work are covered (work instructions for the equipment, work instructions for the preparation of microbiological basis ...), as well as the organizational structure within an institution [4], and technical requirements (equipment, facilities, professional staff ...). Quality records are all records that are created by implementing, maintaining and improving the management system, and include a record of complaints, record of the evaluation contract, a record of the request for analysis, records of nonconforming work, records of purchase request, records of changes to documents, records of correctional and accompanying actions [3, 4].

The quality control system should be traceable connecting all components in the chain of producers of raw materials dehydrated base to supply the documents prepared on microbiological departments.

The documentation of the quality control system defines the responsibilities for individual tasks that are performed within the laboratory: the procurement of necessary chemicals, dehydrated base substrate, antibiotics, antifungal drugs, handling equipment and conduct technological preparation process as well as other factors that affect the final quality of the MM. For the implementation of the quality control system is necessary to ensure trained staff, appropriate equipment and certified reference materials (CRM), with the need to define the working methods and to ensure traceability in the work, to determine the measurement uncertainty, to ensure environmental protection and safety at work [3, 4]. In order to preserve the properties of the MM it is very important to store property dehydrated and prepared media.

By interpreting the results of microbiological tests (food, water, air, soil) that is obtained by using certified base based on the analysis and scientific knowledge come to the risk factors that affect the health of the population.

The main activities for the introduction of a quality system (ISO/IEC 17025:2007) in practice are:

- A high level of quality management (GPM set of rules and procedures to ensure the quality of the process)
- The availability and speed of interpretation microbiologists practitioners
- Expert advice for the use of base
- Daily check background in diagnostics
- Calculate the measurement deviation, inter-laboratory tests.

Thus, the complex requirements of ISO standards in microbiological studies would enable better diagnostics for isolating microorganisms from clinical materials in human medicine, food, water, environment, general use products and medical devices. Standardized media are used to determine the causes of diseases, spoilage and as indicators of hygiene. There is also less risk of errors, as well as financial and time savings in getting the finished results.

It is important to be educated in the importance of microbiological basis to the medical staff who works directly in the activities related to microbiology (bacteriology diagnostics) and the general population, indicating the dangers and consequences of improper preparation and storage of the MM [2]. Improperly prepared MM affects the false negative or false positive findings from samples of human origin, which in turn affects the incorrect setting of microbiological diagnosis and inadequate treatment of the patient. Therefore, part of the bacteriological analysis carried out in the correct selection of antibiotics is used to treat pathogens. This shortens the duration of illness, reduces the occurrence of complications, and it is important to note that improperly or unnecessarily prescribed antibiotics affect the development of bacterial resistance to antibiotics [2].

The quality control system of the prepared microbiological basis is an important traceability in the work and that of the raw material for preparing dehydrated base to the documents prepared to be used for work on the microbiological departments according to DIN EN ISO 11133: 2014 [8]. This is a standard quality control that ensures the quality of MM. All records in the laboratory should be arranged in such a way that it is possible to establish the traceability in an easily verifiable way.

Records of quality control of the prepared documents are all records that are created by implementing technological processes of preparing microbiological basis, control and supervision equipment, and control physical, chemical and microbiological parameters prepared MM.

The procedures used to check surveillance equipment are:

1. calibration and calibration - refers to the control of quantification results after setting the instrument to work under certain conditions
2. validation - means checking general accuracy of the instrument.

Actions that affect the quality of the documents prepared:

- quality of deionized water
- storage conditions of dehydrated base substrate
- quantity weight
- melting and sterilization
- pH
- volume spills
- storage of prepared media [9].

The physical and chemical parameters to be controlled in the prepared MM are: pH, thickness of the plate in a Petri dish, filling volume in the test tube, the color, the clarity, consistency, and of microbiological parameters sterility, productivity and selectivity with certified reference materials (CRM) are controlled. CRM contains microorganisms of stable characteristics that are typical for a particular microbial species, and are suitable for demonstrating the effectiveness of a particular MM, and were purchased from internationally renowned collections [8]. Sources of errors during the preparation of microbiological substrates are lumps in dehydrated substrates, changing of the pH-value, clouding, precipitation, discoloration, contaminated surfaces, weak or superabundant growth of microorganisms, as well as atypical colony growth. Therefore, it is very important to observe the rules of good laboratory practice [9], and to ensure compliance of the technical requirements in the process of preparing MM.

Laboratory for preparation of microbiological base occupies an important role in quality assurance of microbiological results in bacteriological diagnosis. The laboratory is qualified according to the requirements of ISO/IEC 17025:2007 and maintaining the status of accredited laboratory requires continuous care of the qualifications of laboratories and the quality of work performed. The process of systematically kept records documents the technical competence of laboratories, the accuracy and reliability of microbiological test results. All this is to ensure quality services in microbiology with parasitology thereby increasing productivity of bacteriological tests and thus the whole system of microbial activity.

Throughout the disciplinary studies, on the basis of microbiological analysis, based on microbiological substrates produced in quality system (ISO/IEC 17025:2007 and ISO 9001:2000) users, residents of Split-Dalmatia County would be provided with:

- Unquestionable microbiological diagnostics samples of human origin to the unit of local self-government
- Unquestionably monitoring the health of drinking water, water for recreation and physical therapy, sea water, surface water and waste water, water for dialysis, and the hygiene of foodstuffs and general use and monitoring of air quality in the units of self-government

- A quality monitoring of environment and food on the health of the population of local self-government, and based on the results, propose and participate in the implementation of measures to prevent their harmful effects
- Using accurate microbiological diagnosis is performed early detection and suppression of Chronic Diseases is performed and the implementation of measures to prevent, and thus reducing the cost of the employers and the insurance company is suggested.

A prerequisite for accreditation of microbiological methods is a constant quality control of microbiological media (MM) prepared in the laboratory. Testing laboratories determine control procedures of MM prepared from dehydrated base as well as the ways to control technological processes of preparation according to DIN EN ISO 11133:2014. To prepare MM dehydrated bases that are produced in accordance with the wording of the pharmacopoeia (EP, USP) and reference to international standards (ISO, APHA) are used. The dehydrated base substrate MM should be prepared taking into account the principles of good laboratory practice and following the manufacturer's instructions. The records of all stages of preparation and quality control of the MM, ensures the traceability of each issued microbiological finding which is based on the method of cultivation of bacteria.

Microbiological media prepared in the laboratory was founded in order to permanently improve the laboratories quality and competence, which will lead to the development and raising standards through their joint work based on common interest. Joining, networking and inter-laboratory testing facilitate the laboratories entry in EU market. The laboratories are the key element of the infrastructural quality, the trade logistics and the international trade. Without competent laboratory, there can be no reliable product or market. Our laboratories give reliable, credible results, which come from accountability of extensive initial knowledge and additional lifelong education through seminar programs.

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