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# MODERN METHODS OF BACTERIOLOGICAL INVESTIGATION OF THE AIR

[Following is a translation of an article by V. V. Vlodavets in Zhurnal Mikrobiol, Epidemiol, i Immunobiol (Journal of Microbiology, Epidemiology and Immunobiology), Vol. XXX, No. 12, Moscow, 1959, pages 48-54.]

The beginning of biological investigations of the air was founded in the middle of the past century by Pasteur. Since that time, dozens of various apparatuses and methods for study of airborne microflora have been proposed. The intensive development and perfecting of the methods of investigation were closely connected with definite periods of intensive study of the microflora of atmospheric air, air of living and hospital quarters as well as of artificial bacterial aerosols. The latter are utilized more and more for determination of the effectiveness of various means and methods of disinfecting the air and for the study of the epidemiology and pathogenesis of aerial infections.

The presence of a large number of methods testifies to the absence of a single satisfactory method as well as to the possibility for applying various methods for a differentiated approach to the study of bacterial aeroplankton with consideration of its colloid-chemical state (drop and dust phase) and dispersity. It is necessary to note that all ofthe methods of collecting are based on the physical and and colloid-chemical properties of bacteria suspended in the air and, as a rule, do not differ from the method of aerosol catching. The only difference in principle is that in catching bacteria and viruses it is necessary to preserve their viability.

After publication of the monograph of Shafir in 1945, many reports appeared on new apparatuses and methods of investigating airborne microflora and their refinements. Therefore, the aim of this survey is to illuminate the modern methods of investigation of the air, proposed mainly during the past 10-15 years. The earlier proposed methods of investigation are described only when they still find application today (these methods are well described in the Shafir monograph.

All of the methods of bacteriological investigation of the air may be divided into several groups, depending on

the principle of c.lecting the migroorganisms.

1. Qualitative Methods
   A. Method of Precipitation (sedimentation)
   B. Fastening Microorganisms to a Solid Medim
2. Quantitative Methods
   A. Catching Microorganisms on a Solid Medium
      a) centrifugation
      b) slit method
      c) the "funnel" and "sieve" method
      d) electroprecipitation
      e) thermoprecipitation
   B. Aspiration Through Liquid Media
      a) percolation (blown through)
      b) dispersion of the liquid (adsorption on drops)
   C. Filtration
      a) fibrous tissues
      b) membranous filters
      c) solvent filters

The qualitative methods of investigation do not allow one to determine the content of microorganisms in the unit of air volume, while by means of aspirational (quantitative) methods the number of bacteria in a definite air volume can be determined. Further description of the methods will be conducted according to the diagram above.

1. Qualitative Methods

The method of sedimentation, proposed by Koch in the last century, is the simplest method of air investigation and therefore, despite its shortcomings, is utilized to this day. The seeding is performed on open Petri dishes with solid nutrient medium. The microflora of the air, under gravitational attraction, settles on the surface of the nutrient medim or is attached to it by air currents. In subsequent incubation, the viable microorganisms form colonies on the nutrient medium.

By means of the precipitation method it is not possible to determine the number of microorganisms in a definite air volume, since recounting according to Omelyanskiy (on an area of 100 $cm^2$ during 5 minutes the quantity of bacteria contained in 10.1 of the air precipitates) does not give a sufficiently accurate quantitative pictur even for the air of enclosed spaces. As far as investigation of atmospheric air is concerned, recalculations according to Omelyanskiy are absolutely unusable, since the fixing action of air currents

is the basic factor which conditions the degree of seeding of Petri dishes with bacteria. By means of the method of precipitation, furthermore, the finely dispersed fractions of a bacterial aerosol are poorly caught. Therefore, the method of precipitation may be utilized only for qualitative characteristics of air microflora; furthermore, it is necescary to consider that mostly those microorganisms are caught which are connected with large dust particles.

At present, the precipitation method is rather widely applied for investigation of artificial bacterial aerosoles, created in experimental chambers in a study of various methods of air disinfection.

The aeromicrobiological fixative of Matveyev is based on the fixing of bacteria by an air current to a nutrient medium. In this method, the Petri dish is placed at an angle of 45° in a mobile basket together with a vane. With a change of wind direction, the Petri dish turns, presenting the agar surface towards it.

Stoyanovskiy and Reva proposed an apparatus which consist of a funnel and a tube into which the Petri dish is placed. Samples are taken while an automobile moves, so that the air strikes the surface of the nutrient medium with considerable velocity.

Kelly, Pady and Polunin utilized an analogous principle for investigation of air microflora from an airplane. In the aerocentrifuge of Rechmenskiy, the blades of the ventilator are replaced by a plexiglas disc on which it is possible to fix 1 to 4 tubes with cellophane plates, covered with vaseline oil. When the disc rotates, the bacterial particles are glued to the plate, after which seeding by means of impression is performed. The apparatus serves for the qualitative determination of pyogenic flora in hospitals.

In the rotator of Cvetanovic, the dish is placed inside a tube, with a diameter of 4 or 8 cm, which has a special handle. By means of this handle, the apparatus is rotated so that the air current strikes the surface of the dish. In the apparatus of Vasil'yev, the Petri dish is placed at the bottom of a cone with ventilator blades, which is brought into motion manually and which directs the air current to the dish.

All of the enumerated methods can give an idea only o of the qualitative composition of air microflora and mostly of microorganisms connected with large dust particles.

These devices, with exception of the aerocentrifuge of Rechmenskiy, are designed for the investigation of air in the absence of more sophisticated devices or in the absence of electrical energy.

2. Quantitative Methods

The apparatuses which catch bacteria on a solid nutrient medium have the following advantages:

1) seeding of the air on the medium is performed at the time of sample taking·

2) large volumes of air are rather quickly removed (except the thermoprecipitator), and,

3) parallel seedings give rather similar results.

The disadvantages of this group of methods are the need to have a set of media on the object under investigation, the impossibility of isolating the filtrable viruses and rickettsia, as well as the fact that in seeding of one particle or drop which contains several bacteria, one colony grows and, thus, the indexes of the seeding content of the air are decreased.

To these methods belongs centrifugation. The apparatuses of Wells and Shafir are based on the principle of utilizing centrifugal forces for catching bacteria from the air. In the aerocentrifuge of Shafir, the investigated air enters along a tube down to the bottom of a glass cylinder the walls of which are covered with a nutrient medium. In fast rotation of the cylinder (3,000 rpm), the particles containing bacteria are fixed to the agar surface at the time of air passage from the bottom to the opening of the cylinder.

For prevention of medium sliding, 5 percent agar is used which is poured into the cylinder in the molten state and then placed into the apparatus. In fast rotation, agar is evenly distributed on the walls of the container and congeals on them. Before use, the cylinder with agar is dried in a thermostat. The disadvantage of the method is the difficulty in counting colonies inside the cylinder on the background of the opposite layer of agar, as well as seeding off of colonies from the cylinder and their differentiation, since the colonies are observed either from below or through the layer of agar on the opposite side. Recently, Shafir and others, in conducting virological investigations of the air, have been replacing the agar in the cylinder with 20 ml. of buffer solution.

Another in this group is the slit method. The apparatuses based on the slit method are, at present, the most widely used in the entire world. It was proposed for the first time in England in 1942 by Bourdillion, Lidwell and Thomas and was later perfected by Bourdillion with coworkers and

Lidwell. In our country, the apparatus of Krotov* finds wider and wider use. In Poland, the combined apparatus of Lazowski and Kancelarczyk has been proposed; in Rumania, that of Ardelean with coworkers; in the USA, the apparatus of Leckish, Taylor and Hollodey and the slit apparatuses of Decker and Wilson, as well as of Pady; in Czechoslovakia, the apparatuses of Rashka and Shipa, Symona, Braurova, Fisher and Votochek; in Sweden, that of Laurell with co-workers.

The Krotov apparatus, like other apparatuses of this type, is based on the impinging action of the air current. The current of air, passing with a velocity of 15-20 m/sec through a narrow wedge-shaped slit, strikes the medium surface in the Petri dish, as a result of which the bacteria are detained on the moist surface of the agar, placed 2-3 mm below the slit. The Petri dish is placed on a rotating table, due to which the bacteria are rather evenly distributed on the surface of agar. The slit apparatuses are characterized by high catching ability, since the great velocity of the air current fixes considerable quantities of air microorganisms to the agar surface. For selection of air samples in field conditions, Pokrovskiy, Kishko and Ostranitsa have adapted Krotov apparatus for work with direct current from a battery. Under the conditions of sample selection at negative temperatures, Zuykova recommends placing discs of milk glass cloth /?/ in Petri dishes which are lubricated with binding silicone compounds (polysiloxane fluids). After taking the air sample with the Krotov apparatus, the seeding on solid nutrient medium is performed by the impression method.

Let us pause briefly on some modifications of slit apparatuses. Lidwell proposed a slit apparatus for prolonged selection of air samples. Lazowski and Kancelarczyk proposed a universal apparatus, which can catch bacteria on a dense nutrient medium (slit method) as well as in liquids; furthermore, the air which passes out of the apparatus is sterilized by means of filtration. Kuehne and Decker erected a clock mechanism on the portable apparatus of Decker and Wilson with regulation of one rotation of the dish during 1, 2, 5½ and 12 hours. In some apparatuses it is possible, aside from alternating current, to utilize the power supply from batteries.

The "funnel" of Hollaender and Dalla Valle is an apparatus in which the Petri dish is placed under an inverted funnel. The air drawn through the funnel seeds the surface of the agar in the dish. The apparatus is weakly sensitive, since the velocity of air current and, consequently, the impinging force are small. A considerable portion of fine

*At present, the Krasnogvardeyets Factory has started serial production of a new portable model of the Krotov apparatus, which is described by the author in the book Some Problems of Hygiene of Therapeutic Institutions, Leningrad, 1957, 50-54.

bacterial particles and drops are not detained on the surface of the agar and are carried out by the air current. In the Berry apparatus, the funnel method is combined with the method of electroprecipitation - a Petri dish is placed on an electrode, as the result of which the catching effectiveness increases. Symon proposed two apparatuses, based on the "funnel" method - turbine and double funnel aeroscopes. In the latter apparatus the air, consecutively through two funnels, reaches two Petri dishes.

More effective is the sieve method, proposed by Du Buy and Crisp. A Petri dish with the medium is fastened to a small apparatus, in which the incoming air passes through a plate containing about 300 openings with diameter of 0.5 mm. The openings on the plate are distributed in circular rows, due to which the dish is seeded not evenly but in the form of circles corresponding to the openings on the plate. At the present time, this method is rather widely used in investigations of aerial spreading of infections in bacteriological and virological laboratories. The apparatus of Sulica and Antal is also based on the sieve principle.

In the apparatus of Anderson*, the sieve method is combined with the method of a cascade impactor. The apparatus consists of 6 steps on which six Petri dishes are consecutively placed. In the upper step, the openings in the sieve-plate there are the largest; they gradually become smaller towards the last step, so that in aspiration the velocity of the air current which enters on the top dish is smallest and that on the bottom dish of the apparatus is the greatest. As a result, the largest bacterial drops or dust particles are caught in the upper dishes of the apparatus, smaller ones on the middle dishes, and only finely-dispersed fractions of bacterial aerosol on the lower dishes. Thus, this apparatus serves not only for catching microorganisms, but also for determining the dimensions of particles and drops of bacterial aerosol.

Also based on the impact principle is the apparatus of Lidwell which consists of four steps. This apparatus enables one to perform the selection of large volumes of air during a short period and to determine the correlation of various fractions in an aerosol.

The method of electroprecipitation is based on the attraction of charged bacteria to the surface of a nutrient medium in a high-voltage electric field. For this, Petri di-

*I take this opportunity to express my gratitude to Dr. K. Spendlav, who kindly supplied the information regarding the Anderson apparatus.

shes are placed on an electrode to which high-voltage dc is applied.

However, one electroprecipitation does not assure a high degree of catching microorganisms from the air; therefore, in all apparatuses, the principle of electroprecipitation is combined with one of the methods of catching bacteria on the surface of a dense nutrient medium, i.e., a Petri dish is placed in the electric field and the investigated air is supplied to the surface of the nutrient medium. Thus, in the apparatus of Berry, the electroprecipitation is combined with the "funnel" method; in the apparatuses of Thompson, and Rechmenskiy, Ruks and Mesonnae, with the impact force of the air current; in the apparatus of Uspensky and Lebedev, with the method of precipitation.

In the complex and sophisticated apparatuses of Lekish, Taylor and Holodey, Symon, Binek, bacteria are caught simultaneously on two titer dishes which are placed on the positively and negatively charged electrodes. Vershigora proposed an electroprecipitator in which bacterial particles and drops are charged unipolarly (negatively) by means of a corona discharge and the catching of the bacteria is performed on the electrode which has the opposite (positive) charge. Since in all such apparatuses two principles of microorganisms - catching act, they, as a rule, assure a high degree of catching microflora of the air.

The principle of thermoprecipitation is based on precipitation of particles from warmed air in contact with a relatively cool surface, which was used by Kethley, Gordon and Orr for catching bacteria from the air. Later, a more perfect apparatus was created - the thermopositor (Orr, Gordon and Kardecki), in which the investigated air passes between surfaces heated to 125° and cooled to 25°. On the latter, there is located filter paper saturated with 1.15 percent agar, on which bacteria are caught. After taking the air samples, the paper is placed into nutrient agar. The disadvantage of the apparatus is the low velocity of air suction (0.3 l/min) and the fast drying of the agar on the filter paper.

The methods based on aspiration through liquid media have the following advantates:

1) In taking samples and in seeding, the bacterial particles or drops are dispersed and, thus, the number of captured microorganisms increases and becomes more exact;

2) the liquid may be investigated for virus and rickettsia content;

3) from one volume of liquid, seeding on several dishes

with various media may be performed as well as infection of laboratory animals.

The disadvantage of this group of methods is that the seeding of a small part of the fluid in recalculation to 1 $m^3$ of air requires multiplying by large factors, which decreases the accuracy of investigation; the speed of sample taking is considerably smaller than on solid media. In seeding of the liquid on dense nutrient media, creeping growth of gram-positive bacilli is sometimes observed; in negative temperatures the liquid freezes quickly and these apparatuses are fragile (glass).

The simplest method utilizing liquid media is the apparatus of D'yakovlev. It is a cylinder into which, through a stopper, two tubes are introduced. One tube is submerged in the liquid, the other ends a little below the stopper. The air is sucked through the liquid by means of an aspirator. In the modification of Milyavskaya, at the end of the tube, there are 15-20 small openings in order to break up the air current into smaller bubbles and by this to increase the surface of tis contact with the liquid. In the capillary impinger of Roseberry, the end of the tube ends with a capillary, serving the same purpose.

For choking, Shafir utilized the Drexel flask; Lemon, the Folin tube from the apparatus of Van Slayk for determination of urease; Cvetanovich, a 70 ml test tube; Fomin, the narrowed part from a Libich's cooler; Kiktenko with collaborators, a U-shaped tube with a large number of glass beads. After taking the air sample, the seeding of the fluid is performed (physiological solution, tap water, broth) to a solid nutrient media. Chaykovskiy and Ugorskiy utilized a "rinser" in the form of a cylinder with a narrowed bottom, into which the fluid which, by means of rotation, is evenly distributed on the cylinder's surface.

The bubbling apparatus of Vershigora is a cylinder into which are soldered two glass plates with 30 openings in each. Due to small openings in the bubbling apparatus, a high degree of contact of the air with the catching fluid is achieved.

To the methods which are based on the dispersion of fluid belongs the bacteriocatcher of Rechmenskiy, the action of which is based on the atomization (dispersion) of the fluid by the current of passing air and the catching of the bacterial particles and drops suspended in the air on drops of the liquid. Into the receiver of the apparatus, sterile broth or buffer solution is poured, which in samples taking is sucked out of the receiver and is dispersed into drops by the air-current force.

These drops hit the barrier - a glass strip - and are dispersed into still smaller drops. Thus, in the apparatus,

a small cloud of rather small drops is created on which, according to the principle of adsorption, bacteria in dust and drop phases of bacterial aerosol become caught. The great advantage of the apparatus is its ability to catch the very small and fine bacterial drops which are poorly caught by other apparatuses.

The drops of the fluid are detained on the walls of the apparatus and flow back into the receiver. After sample taking, seeding (on dense media) and other investigations are performed.

Lately, the apparatus has been successfully utilized for virological investigations of air by Vizitiu and Galikeyev. As Labinskaya demonstrated, the bacteriocatcher of Rechmenskiy possesses high sensitivity in respect to capturing pathogenic microflora in the air.

The apparatus of Moulton, created in accordance with the same principle, is also highly effective. However, its construction is considerably more compled. In view of the complexity of its construction and the difficulty of working with it, the apparatus of Multon did not find wide application. Later the apparatus was simplified by Albercht, who aseembled it from three removable glass parts; however, the apparatus remained rather complicated.

The methods based on filtration of the air have the following advantages:

1) they are applicable to bacteriological investigations of the air in negative temperatures;

2) possibility of performing seedings on media through various time intervals after sample taking;

3) relative speed of sample taking;

4) its usability in separate cases for virological investigations of the air.

Advantages and drawbacks belong to the various filters that correspond to those in the methods of catching aerial bacteria on dense or liquid nutrient media. Thus, the disadvantages of fir bus and dissolving filters have much in common with the disadvantages of methods of catching on liquid media, while in membranous filters they are close to the disadvantages of the methods of catching bacteria on dense nutrient media.

Fibrous filters have been used for a long time in bacteriological investigation of the air. Cotton filters were utilized before by Frankland and Mats. Glass wool filters

were used by Mats. For this goal, Nasledysheva and Miroshnikova utilized paper filters which, after taking the air sample, were softened in water. Nakhinson, Katsnel'son and Gorodetskiy propose a cartridge with six layers of gauze, which is later washed off with physiological solution. Besson and others utilized paper-asbestos filters in an investigation of the microflora of Paris, and Torloni and Borzzani used Wattman filter paper Nos 40 and 42.

A simple method of investigation was proposed by Zubarev. Into a metallic adapter, a loose piece of hygroscopic cotton is placed and sterilized in this state. The taking of air samples may be performed with any aspirator. The author recommends using a manual pump with a capacity of one liter, which alloes applying this method under conditions where there is no electrical energy. After taking air samples, the cotton is washed off in 2-3 portions of physiological solution and the obtained suspension is seeded on membranous filters. For investigation of air microflora from airplanes, Skrzhiskaya utilized filters of Japanese filter paper, and Kelly, Pady and Polunin used filters of glass wool.

Fibrous filters retain considerable quantities of aerial bacteria. However, it is not possible to wash off all bacteria adsorbed by the fibers; therefore, a considerable part of bacteria caught by the filters is not determined in seeding on dense nutrient media. Besides, getting separate fibers in subsequent seeding on the surface of membranous filters or agar leads to multiplication of bacteria along the fibers, which frequently conditions the total growth.

Membranous filters were utilized for the first time for bacteriological investigation of air in 1937 by Reznik and later were used by Korchak-Chepurkovskaya (1941) and Milyavskaya (1947). Abroad, the membranous filters were utilized for this purpose later - in Germany in 1948 by Krause, and in 1957 by Albrecht, in 1953 in the USA by Goetz, and in 1955 by Sery and Gizovo in Czechoslovakia. The principle of catching consists in detaining the bacteria suspended in the air in narrow passages of the filter and in subsequent seeding of membranous filters on a nutrient medium. Some authors feel that the electric charge which forms on the surface of the membranous filter when air passes through it plays a greater role in detaining particles and aerosol drops.

Before use, the membranous filters are sterilized by bo boiling two times for 15 min. each, and then are dried in a thermostat in a sterile Petri dish between two layers of filter paper for 24 hours. For air investigation, membranous filters Nos 2, 3 and 4 of the Mytishchenskoy Membranous Filters Factory are used.

These filters are designed for bacteriological investigation of water and possess rather low air-conductance; therefore, for taking air samples, rather powerful air blowers are necessary. In taking samples of atmospheric air, membranous filters should be guarded against liquids (rain, snow, condensed water) since a wet membranous filter is practically airtight.

In the apparatus proposed by Gal'per and Ruban for catching pathogenic bacteria and filtrable viruses from the air, membranous filters and the Dyakonov apparatus are combined. The authors feel that bacteria are detained on the surface of a membranous filter at the time when viruses penetrate through the membranous filters and are detained in the liquid of the Dyakonov apparatus. The air aspiration is performed by means of an automobile engine.

Water-soluble filters have been widely developed during the past few years. Mitchell and collaborators proposed and perfected filters of soluble gelatin foam. Kajiwara and Samori and later Vanini developed and perfected filters of sodium glutamate, Richards of sodium alginate and Hammond of ammonium alginate.

In our country, methods of preparing soluble filters of gelatin foam were developed by Vershigora. Filters of this type differ in principle from those in use in the past century made of soluble salt ($Na_2SO_4$, NaCl, $MgSO_4$) and sugar in that they do not induce a sharp increase of osmotic pressure when dissolving in water, i.e., they do not induce a toxic influence on microorganisms caught from the air.

After taking air samples, the filters are dissolved in a definite volume of sterile water or physiological solution. All further investigations are conducted in the same way as in catching microorganisms in liquid. The positive aspect of soluble filters is the possibility of utilizing them for virological investigations of the air (Rechmenskiy, Mitchenk), as well as of conducting bacteriological investigations of the air in negative temperatures.

It should be stressed in conclusion that the number of methods utilized for bacteriological investigation of the air is rather large and continues to increase with each year. With a consideration of positive properties and disadvantages of various methods, this lets one select the most convenient and effective method for this or that investigation.

It was established on the basis of a comparative study of a number of methods that the most effective apparatuses for bacteriological investigation of the air of buildings (dust phase of aerosol) are the bacteriocatcher of Rechmenskiy and the Krotov apparatus (Vlodavets).

Then, in order of decreasing effectiveness of catching,

follow the membranous filters, the apparatus of D'yakonov, the aerocentrifuge of Shafir and the method of Zubarev.

The bacteriocatcher of Rechmenskiy is more suitable for discovery and isolation of pathogenic bacteria and viruses from the air. For conducting standard hygienic investigations of the air (determination of general bacterial seeding and content of sanitary-indicating microorganisms) it is possible to use the Krotov apparatus. For investigation of the air at negative temperatures, the most useful are membranous filters which are not subject to the influence of low temperatures. Under experimental conditions the viruses dispersed in the air may be caught in a liquid medium by means of the bacteriocatcher of Rechmenskiy, the aerocentrifuge of Shafir, the apparatus of D'yakonov as well as by means of water-soluble filters and, in particular, filters of gelatine foam.

It should be kept in mind that drops and dust particles of various dimensions are not caught by different apparatuses in the same degree. Besides, the dust and drop phases of bacterial aerosol are also caught differently by different apparatuses. As a rule, the finely dispersed fraction of bacterial aerosol in the drop phase is less readily captured than is the large-dispersed. These differences in the effectiveness of catching of various phases and fractions of bacterial aerosol are mostly at the basis of the differing results which are obtained by individual authors in evaluations of the catching ability of the same apparatuses.

At the present time, the task of aeromicrobiology is the development of faster methods of bacteriological investigation of the air and of methods of isolating filtrable viruses from the air. Further perfecting of the available methods of bacteriological air investigation is necessary and, in particular, the development of effective methods of investigation of atmospheric air under field conditions as well as methods of investigation under negative temperatures.

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