# Guest Forum

## The Screening Committee Lecture for the First Dr. Masao Horiba's Award

# Clinical Examinations and Treatment of Infections

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**What are the application possibilities of analyzers in the medical field? This report discusses the applications of pH measurement are foreseen to be as diagnostic aids for patients with serious diseases such as bacteremia, and the actual statuses of the Central Clinical Laboratory and the Infection Control Team that are the cutting-edge areas in the interventions of infections.**

## Introduction

The examination of infectious diseases may be the least developed field in clinical examinations from the viewpoint of objective and technical sensing. Examinations of infectious disease in the clinical field require visual checks of bacteria developed in cultures after waiting for living cells to divide. It always takes at least one day and even up to one month depending on the types of bacteria being analyzed as a result of an examination.

## Flows and Purposes of Diagnoses and Treatments of Infectious Diseases

In the diagnosis and treatment of infectious diseases in the clinical field, physicians examine patients, clinical technologists obtain the specimens such as sputum and blood, etc of the patients and the physicians diagnose and treat the patients based on the results. The bacteriological testing on this occasion has the following two purposes:

- Identification of offending bacteria
- Prescription of an appropriate drug for the bacteria

The two items are executed as fast as possible and at the lowest cost in the most correct manner. However, it is very difficult to determine which is the offending bacteria and prescribe a suitable drug for a patient because there are many types of bacteria and drugs. The four methods to isolate bacteria include (1) smears (an examination under a microscope), (2) culture (an inoculation on a culture), (3) observations of antigens and (4) observations of genes. The methods used to observe antigens or genes have been in use for about the last thirty years. The smear test and culture examination have been used for hundred years.

The method to observe genes is actually speedy, however it is costly. In addition, genes are not enough because the test of drug sensitivity resistance and infection route identification require living bacteria. Eventually, processes to visually check, inoculate and cultivate bacteria are necessary for observing bacteria. It has been a request to promote the efficiency of these processes.

## Disagreement between the Purpose to Develop Devices and Clinical Tastes

An example of actually developed examination apparatus will now be discussed. Consider that there is a device to find tubercle bacilli, and a device to find methicillin-resistant Staphylococcus aureus (MRSA) that causes nosocomial infections. Which is more in demand, or sells well? Most people may think that the MRSA devices sell well. Regrettably, devices to detect tubercle bacilli are in high demand. Tuberculosis can be diagnosed immediately when even a single tubercle bacilli is found in a specimen. Meanwhile, staphylococcus that causes MRSA is an indigenous microbiota that exists everywhere. Therefore, detecting staphylococcus does not identify a specific disease.

Further, there is a premise that a tubercle bacillus divides itself very slowly and it takes almost one month to identify tubercle bacilli by cultivation. Every year globally, three million die from tuberculosis and ten million develop tuberculosis. It is widely considered that many people become infected with MRSA one after another at hospitals. However, infection from MRSA is absolutely a disease in cities of advanced countries. Not so many devices to detect MRSA are wanted in the world market. This is an example that clearly represents the gap between the issue in the field and the common image of medical care. It should be noted that the understanding of infections and bacteria, and sophisticated clinical sense are required for developing clinical testers.

## Criteria to Determine “Offending Bacteria”

Determining whether a bacterium detected is an “offending bacterium” or not requires the following conditions.

- Detecting a bacterium (irrespective of bacterial types) in tissue (blood, spinal fluid, etc) that should be normally aseptic
- Detecting a non-indigenous bacterium (tubercle bacillus , typhoid bacillus, etc) that does not exist in the human body (irrespective of tissue type)

These two criteria are “clinical senses”. However, in the field, an offending bacterium can rarely be identified so clearly by these two criteria. In most cases, an offending bacterium is identified by detecting bacteria from materials with indigenous bacteria mixed in, Gram staining methods, and comprehensive diagnostic imaging. This unclear region requires a sensor technology that is fast, inexpensive - and correct.

## The Blood Culture Test Procedure

In the case of blood that is aseptic, the same specimen is collected in a bottle containing oxygen (Figure 1) and in a bottle with no oxygen. They are then impregnated into liquid mediums.

Figure 1 Detection of Bacteria by Blood Culture pH Detection

Microbes include anaerobes that dislike air and aerobes that like air. Therefore, a microbe is classified into either one of them first. The pH indicator is used herein. If the bacteria develop, the pH lowers, resulting in color changes of the reagent. The changes are visually observed. In Figure 1, the color of the bottle containing air changes from green to yellow showing that aerobes typified by Bacillus coli, staphylococcus, etc are growing. It normally takes about one day to cultivate the bacteria to confirm the change in colors. However, if the blood culture is positive, the patient will be in critical condition. The patient may die in the one day required for the test if no therapy at an early-stage is performed.

A sensing technology that analyzes a specimen within several hours by utilizing not only changes in colors but also some other appropriate indicators, can save the lives of many patients.

A specimen is taken out of a bottle with a color changed in Figure 1 and Gram stained.

The specimen is examined microscopically. If the specimen is dyed blue, Gram positive coccus is identified (Figure 2). The only thing understood here is that Gram positive coccus is dyed blue by the Gram staining method. The correct name of the bacteria cannot be specified. Possible bacteria include not only gram-positive bacteria but also Gram negative bacillus and larger sized candida. Various procedures such as determining the size of a bacterium visually and catalytic reactions are required to specify the name of a bacterium. For that purpose, in some cases, a bacterium is cultivated to make colonies requiring another day to identify the name of the bacterium. Thus, faster determination is also required in this phase.

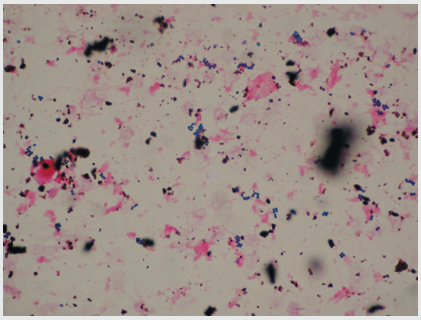

Figure 2 Gram Stained Gram Positive Coccus in the Blood Culture Solution

## Non-indigenous Pathogenic Bacteria

Non-indigenous pathogenic bacteria that do not exist fundamentally in humans will now be discussed.
The Ziehl-Neelsen stain (Figure 3) shows the existence of acid-fast bacteria that have a strong resistance to acids, alkalis and alcohol. For example, tubercle bacillus is an acid-fast bacteria. It is possible to identify a tubercle bacillus by performing PCR (polymerase chain reaction) immediately after detecting an acid-fast bacterium.

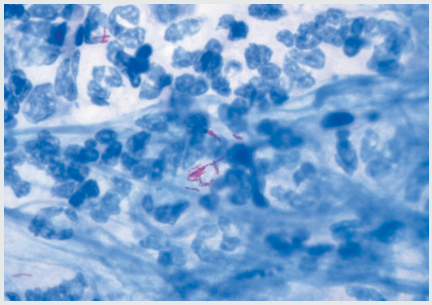

Figure 3 Ziehl-Neelsen Stain

Especially, multiple drug resistant tubercle bacillus is a current worldwide problem. In Japan, it accounts for 0.8% of new tubercular patients. It may seem to be a low percentage, but it is four times higher than five years ago. Particularly, AIDS patients often become infected with this tubercle bacillus. 80% of AIDS patients who become infected with multiple drug resistant tubercle bacillus die within eight weeks.
It is said that the number of AIDS patients will increase in Japan, so it is important to be able to detect the tubercle bacillus. In any case, the current test method is also by a cultivation which requires several weeks to complete.

## Test of Materials Mixed with Resident Microbiota

A procedure to test materials mixed with difficult-to-identify resident microbiota.
If staphylococcus aureus is detected in coughed-up sputum for example, in the case of tubercle bacillus, the infecting organism is identified only with the existence of a bacterium in a blood culture. However, a staphylococcus detected in sputum coughed-up by a pneumonic patient cannot be identified as the infecting organism. That is because staphylococcus is an indigenous bacterium that always exists in the mouth. At first, sputum (a specimen) is evaluated with the naked eye. The patient has difficulty breathing and produces saliva and thick sputum. A staphylococcus detected in a saliva sample (Figure 4(a)) cannot be used as valid data. Meanwhile, an additional test will be performed on a bacterium obtained from a purulent specimen as shown in Figure 4(b). This is also primarily determined by “visual observation”.

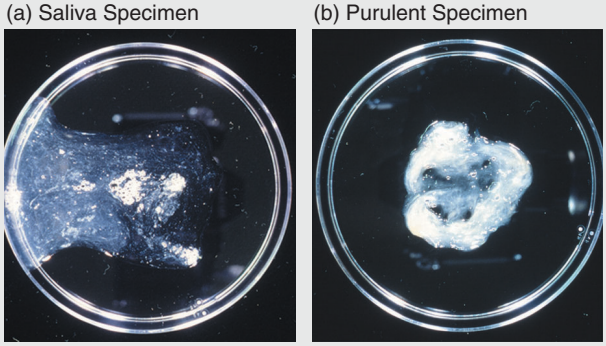


Figure 4 Macroscopic Examination of Specimen

The quality of a specimen is then assessed by the Gram staining method with a microscope (Figure 5). The specimen shown in Figure 5(a) includes a large amount of original squamous epithelium. Original squamous epithelium exists in the mouth not in the lung. It shows that the specimen has not been obtained from a lesion of pneumonia. Meanwhile, the specimen shown in Figure 5(b) includes nothing other than white blood cells showing that it has been obtained from an infected lesion. This is also visually determined.

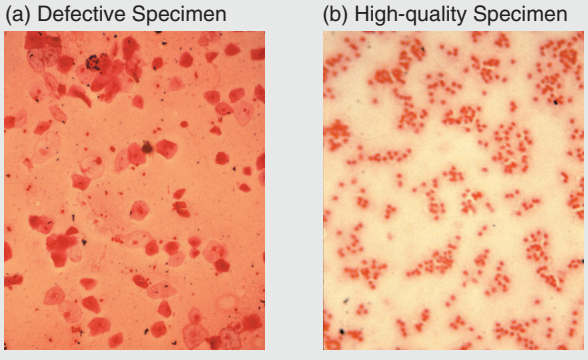


Figure 5 Gram Staining (× 100)

The magnified views of these two specimens show a defective specimen with varied bacteria grown around the original squamous epithelium (Figure 6) and a high-quality specimen with only specific bacteria existing (Figure 7). The high-quality specimen has only staphylococcus around white blood cells. They can be identified as the staphylococcus of an infectious disease. If they are cultivated, varied bacteria grow on a defective specimen (Figure 8). Meanwhile, only staphylococcus grow on a high-quality specimen (Figure 9).

In fact, we identify an infecting organism using such a method. These activities are performed via visual observation. Specimens used in the clinical field are mostly coughed-up sputum, stool, and urine. They are all classified visually.

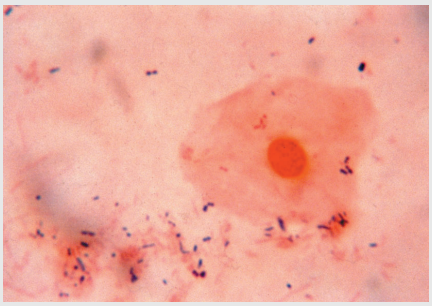

Figure 6 Gram Staining of a Defective Specimen (× 1000)

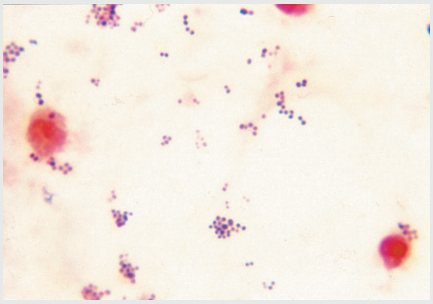

Figure 7 Gram Staining of a High-quality Specimen (× 1000)

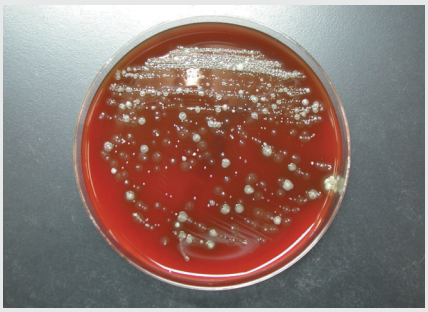

Figure 8 A Culture Result of a Defective Specimen (Indigenous Bacterial Flora)

Figure 9 Culture Result from a High-quality Specimen

## Drug Determination and Test of Drug Sensitivity Resistance

After identifying a bacterium, a test is required to determine a medication to stunt the growth of the bacterium, i.e., to cure the disease. In the clinical field, medications are determined in accordance with the recommendations of the NCCLS*1 all over the world.

*1: The National Committee of Clinical Laboratory Standards or NCCLS was originally a national organization. It has evolved into an International Organization for Standardization but is still called the "NCCLS".

There is a huge range of antibacterial agents. The NCCLS announces data of antibacterial agents in the order of safety, reliability and cost. We determine whether a drug is effective based on these priorities.

Figure 10 shows a test of drug sensitivity resistance. This test method is also a primitive one where bacteria are applied to a culture medium, a piece of paper impregnated with an antibiotic is put on the culture medium and a diameter of the bacteria whose growth is suppressed is noted. The NCCLS defines a larger growth-inhibitory zone to be "S" (sensitive) and a smaller or no growth-inhibitory zone to be "R" (resistance). However, the number of millimeters specified only allows determination of whether an antibiotic is effective or ineffective. The in-vitro concentration and dose of an antibiotic suppressing the growth cannot be confirmed.

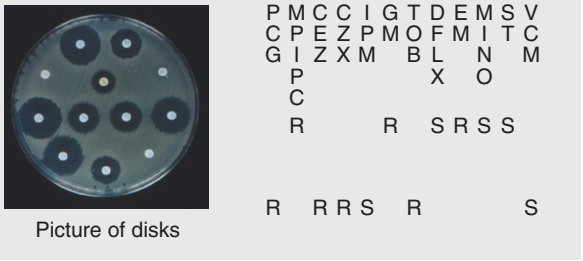


Figure 10 Test of Drug Sensitivity Resistance

As shown in Figure 11, test tubes are lined up in a matrix with dried antibiotics applied in advance at the bottom of each. Each column has test tubes containing the same antibiotic at different concentrations. Types of antibiotics are different from column to column. The concentration of the center tube is 1 μg/mL. The concentrations are 1,4,8 and 16 from the center to the top, and 0.5, 0.25, 0.125 and 0.06 μg/mL downward. A is injected into to them. The minimal inhibitory concentration (a dose of an antibiotic that is effective against a bacterium) can be determined by introducing a certain amount of bacterial emulsion on them.

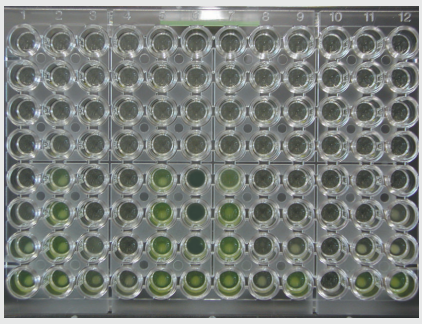

Figure 11 MIC Measurement by Broth Microdilution Method

Figure 11 shows a case of Pseudomonas aeruginosa. The minimal inhibitory concentration of the antibiotic to the bacterium is reported to be 1 if the growth is prohibited at 1 μg/mL after they are left at rest for one day. However, in this method, the turbidity of the solution is visually determined after a day (This method also relies on the human eyes). The doubling time (the time required for a microorganism to double through cell divisions) of Pseudomonas aeruginosa is about 10 to 20 minutes. It is supposed that the composition such as the pH of the culture medium changes in that period. Two purposes including (1) identification of a bacterium and (2) determination of a drug is established by performing these tasks. It takes one day to identify a bacterium and determine a drug respectively. Therefore, the total time period required for this method is two or three days. Measuring and detecting in a shorter period will bring about a great improvement.

## Activities of Infection Control Team, Kyoto University Hospital

The situation of infection control in the current medical field will now be discussed.

A nosocomial infection is a disease spreading around a hospital. That is a big problem at hospitals all over the world as well as in Japan. Pharmacists, physicians, nurses and laboratory technicians cooperatively make infection control teams. That is the trend of the medical care system in the world. Kyoto University Hospital made an infection control team for the first time in Japan though a little late. There may be no other medical facilities other than Kyoto University with the optimum conditions.

Figure 12 is a structure for activities of the Infection Control Team (ICT).

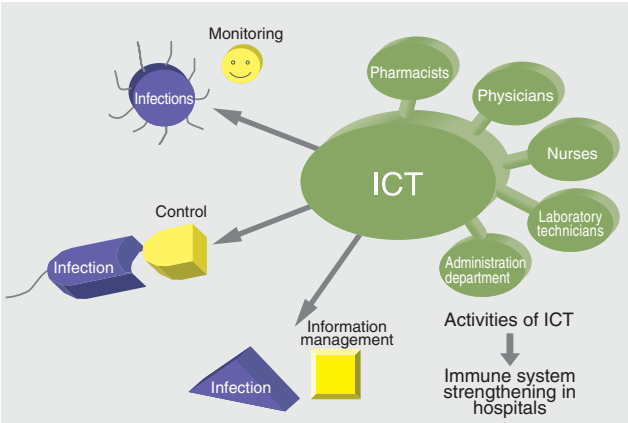


Figure 12 A Structure for Activities of the Infection Control Team

Currently there is a large gap between our infection control team and general anti-infective therapies in Japan.

Infectious diseases occur in every department such as surgery, internal medicine, and pediatrics. However, no department has infectious disease experts. The actual state is that physicians in each department control infectious diseases by watching others' examples. In this situation, it is important to make an infectious disease expert team covering all departments. Our infection control team has

no special building. We examine and treat surgical-wound infections in the surgery department and post-transplantation infections in the internal medicine department.

Actually, Japanese people have little interest in infectious diseases. Many physicians perform ineffective therapies or diagnose wrongly resulting in bad prognoses. We require examination data to predict an infection based on an abnormal result in a bacteria test.

Ten staff in our infection control team check the results of bacteriological testing on computers every morning. Staff visit patients in each department if infectious diseases are suspected.

Recently we visited a baby who was in a state of shock and apparently dying. The attending physician at the Department of Obstetrics and Gynecology did not know what to do.

A very rare bacterium called Campylobacter was found in blood that should be essentially aseptic. Here was a case that couldn't have been treated by anyone other than infectious disease experts like us.

Figure 13 is a list of the results of our activities in a year. We highly value the results of blood culture testing. That is because an infection is identified from a bacterium obtained from blood. This accounted for almost 50% of the total of 636 cases. It is most important to diagnose an infectious disease from a test result. Of course we diagnose infectious diseases with varied testing other than blood culture testing.

Jan. 2002 to Dec. 2002 Total of 636 cases

| | |
|---|---|
| ■ Test result | 500 (79%) |
| Blood culture positive | 286 |
| Other sterile specimen positive | 88 |
| Antibiotic-resistant bacteria | 47 |
| Abnormally high level (ß DC/ET) | 32 |
| Special result | 18 |
| Special bacteria | 17 |
| Acid fast bacteria | 8 |
| Antigen test positive | 4 |
| ■ Consult | 98 (15%) |
| From physicians | 97 |
| From nurses | 1 |
| ■ From medication history | 28 (4%) |
| ■ Others | 10 |

Figure 13 Results of IDC Activity

Surgical cases accounted for the largest number of the total cases. More particularly, implant surgery accounted for the highest number of all departments where many post-transplantation infectious diseases occurred (Figure 14).

(629 cases)

| | | | | |
|---|---|---|---|---|
| Age | : Median | 57 years old | (0-91) | |
| Sex | : Male / Female | | 339/290 | |
| Medical treatment fee | : Surgery department "Except implant surgery department" | | 258 | (41%) |
| | Internal medicine department | | 199 | (32%) |
| | Implant surgery department | | 122 | (19%) |
| | Pediatrics department + Immature infant center | | 54 | (8%) |
| Patient background | : Tumor bearing patient | | 275 | (44%) |
| | Within one month after operation | | 169 | (27%) |
| | Post-transplantation "Hematopoietic organ, liver" | | 131 | (21%) |
| | With CV catheter insertion | | 237 | (38%) |

Figure 14 Breakdown of Cases

It is said that the incidence rate of infections accompanying liver transplantations is 50% or more in the world. As just described, infectious diseases occur in high-risk conditions such as transplantations, cancers and surgeries. In many cases they are very serious. The fatality rate is also very high. 30% of patients with a positive blood culture testing result will die within a month even at medical facilities providing world's most advanced medical treatments. It means that prognoses of patients depend absolutely on early detection of positive blood cultures and appropriate treatments given.

There were 471 cases of infectious diseases in a year. Recurrent diseases in our treatments included postoperative peritonitis: 96, infections due to indwelling catheters: 84, pneumonia: 75, cutaneous infections: 36, urinary infections: 30. Among them, bacteremia has a high fatality rate, however it can be cured with effort. The infection routes of 291 cases of bacteremia with bacteria intrusion into blood included blood infected with bacteria via catheters: 21% (largest rate), postoperative peritonitis: 14%, cutaneous infections (abscessed wounds causing infections): 11%, and infections from artificial respirators: 8%. They account for as much as about 50%. In fact, they are commonly caused by skin punctures from medical intervention. Contaminated catheters must be extracted immediately - though catheters are required. However, in practice, we can not determine that a catheter is contaminated with bacteria unless a patient becomes ill.

For example, a catheter with a function to light-up or change color when bacteria intrudes would indicate the time has come to extract it.

However, in reality, a fever developed from a catheter inserted is diagnosed as “catheter fever”. In many cases, physicians consider that extracting a catheter corrects the problem. However, bacteria have already intruded into the body. Therefore, it is required not only to extract the catheter but also administer the correct antibacterial drugs. Many physicians lack such a basic awareness because they do not understand the importance of basic testing. It is required to establish technologies to detect bacteremia earlier and determine appropriate medicaments rapidly.

We cannot administer an optimum dose of appropriate antibacterial drugs if it takes a few days to test drug sensitivity resistance.

As discussed, we have guided and intervened in various departments by identifying bacteria in cases where physicians are unfamiliar with infectious diseases and have determined effective drugs and their doses based on testing. As a result, useless drugs, medical treatments and testing are decreasing.

Figure 15 shows the results of the interventions.

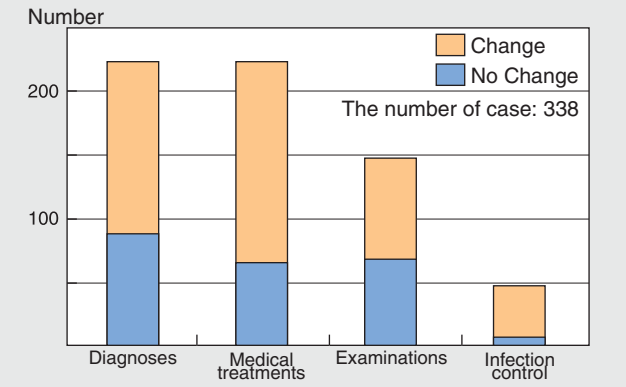


Figure 15 Results of Interventions

The Japanese Fee-For-Service health-insurance is a system where all costs of medical treatments performed are paid. However, Kyoto University Hospital has adopted the fixed charge system for medical treatment fees. The fixed treatment fee for diagnosed pneumonia is 500,000Yen. No one pays more than 500,000Yen no matter how many treatments are tried. In this system, all useless examinations are loss making.

Therefore, we shall perform useful examinations to eliminate the number of useless examinations. What is important is the effectiveness of examinations.

As shown in Figure 16, the number of cultivation tests of throat saliva obtained from patients’ is dramatically decreasing. Meanwhile, the number of meaningful examinations in urgent need such as blood cultures is increasing (Figure 16).

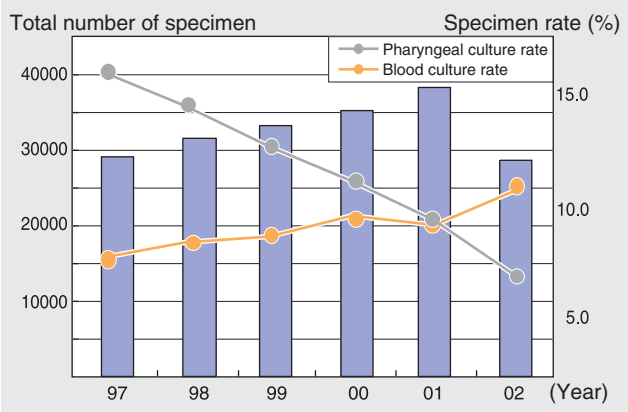


Figure 16 Guidance and Intervention for Diagnosing Infectious Diseases

The mortality rate of life-threatening illnesses is being significantly decreased by our activities. We can save patients’ lives while reducing waste. There are an increasing number of physicians in every department who ask for our advice. We believe that it is because our proven practical ability to save patients’ lives has become highly valued.

## Conclusion

Physicians and medical technicians could diagnose critical patients at an earlier stage and securely save their lives at lower cost by using the technologies of medical-equipment companies. We believe that it contributes to the improvement of Japanese medical services. The sophisticated skills of Horiba, Ltd. by enhancing the effectiveness of our visual observations will further propel the progress of medical science.

<Extracted from the Screening Committee Lecture for the Dr. Masao Horiba’s Award (July 7, 2004)>