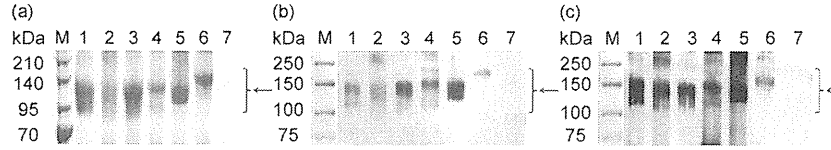

FIG. 2. Identification and characterization of purified His-GPs. (a) His-EBOV-GP and His-MARV-GP were analyzed by 8% SDS-PAGE and stained with Coomassie brilliant blue. (b and c) Immunoblotting of purified His-GPs was performed by using MAbs to EBOV (ZGP42/3.7) and MARV GPs (AGP127-8) (b) and His tags (c). Arrows indicate the locations of the His-GPs. The protein bands represent His-ZEBOV-GP (lane 1), His-SEBOV-GP (lane 2), His-CIEBOV-GP (lane 3), His-BEBOV-GP (lane 4), His-REBOV-GP (lane 5), and His-MARV-GP (lane 6). Lane 7 shows FCS-derived proteins used as a control antigen (see Materials and Methods).

was approximately 0.01 to 0.1 μg/ml. On the other hand, ELISA using membrane lysates of GP-transfected cells or VLPs under similar conditions with the GP-based ELISA showed lower sensitivity, except for the Angola serum and VLP combination (Fig. 3d to i). This is most likely due to the interference by the residual detergent and/or irrelevant proteins in the lysates and VLP antigen preparations.

Specificity of the GP-based ELISA. Next, the species specificity of the ELISA was assessed by testing the antisera of mice immunized with VLP containing the respective EBOV and MARV GPs. We found that species-specific IgG antibodies were clearly detected in these mouse antisera (Fig. 4a to f). All the anti-EBOV IgG antibodies in the sera showed low reactivity to heterologous EBOV GPs, and no cross-reactivity to MARV GP was found (Fig. 4a to e). Similarly, anti-MARV VLP serum antibodies reacted to MARV GP but not to EBOV GPs (Fig. 4f). These results indicated that this purified GP-based ELISA sufficiently detected filovirus species-specific antibodies. On the other hand, the VLP-based ELISA was less sensitive and detected more appreciable cross-reactive antibodies in some of the mouse sera, likely specific to NP and VP40, than the purified GP-based ELISA (Fig. 4g to i).

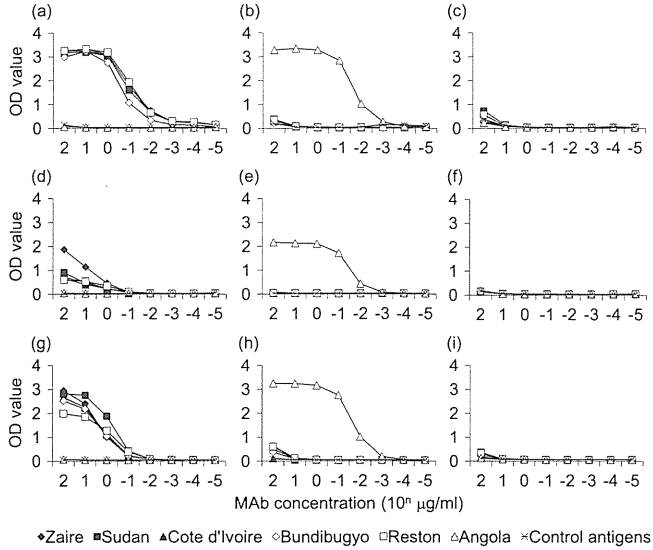

FIG. 3. Sensitivity of ELISAs. His-GPs (a, b, and c), GP-expressing cell lysates (d, e, and f), and VLP (g, h, and i) were used as antigens. The GP amounts were standardized by Western blotting as described in Materials and Methods. Serial 10-fold dilutions of MAbs to EBOV (a, d, and g) and MARV (b, e, and h) were prepared and tested. S139/1 (specific to influenza virus hemagglutinin) was used as a negative-control antibody (c, f, and i).

Analysis of clinical samples in the GP-based ELISA. To further confirm the specificity of our ELISA, we used convalescent-phase plasma samples obtained from monkeys experimentally infected with ZEBOV or SEBOV (Fig. 5). The cutoff OD values (i.e., the mean plus 3 standard deviations of the five negative serum samples) were 0.23, 0.22, 0.29, 0.22, 0.17, 0.20, and 0.13 for His-ZEBOV-GP, His-SEBOV-GP, His-CIEBOV-GP, His-BEBOV-GP, His-REBOV-GP, His-MARV-GP, and control antigens, respectively. According to these thresholds, all infected monkey serum samples tested were EBOV antibody positive. We detected IgG antibodies in the ZEBOV-infected monkey plasma with higher reactivity against His-ZEBOV-GP than against any heterologous GP antigens. Although IgG antibodies in the SEBOV-infected monkey plasma showed binding to all His-EBOV-GPs, the highest reactivity was observed with the homologous antigen His-SEBOV-GP. Neither of these plasma antibodies reacted with MARV GP.

We then examined IgG antibody levels in serum or plasma derived from ZEBOV-, SEBOV-, and MARV-infected patients (Fig. 6a). The cutoff OD values obtained from the five negative-control sera for IgG antibodies were 0.20, 0.17, 0.24, 0.18, 0.14, 0.27, and 0.23 for His-ZEBOV-GP, His-SEBOV-GP, His-CIEBOV-GP, His-BEBOV-GP, His-REBOV-GP, His-MARV-GP, and control antigens, respectively. For most of the samples tested, IgG antibodies to homologous GP antigens were detected with the highest reactivity (Fig. 6a). All of the samples derived from ZEBOV-infected patients cross-reacted with His-CIEBOV-GP and His-BEBOV-GP antigens, whereas only one of the SEBOV-infected human samples (sample 9) showed cross-reactivity with His-MARV-GP. Overall, the level of cross-reactivity was consistent with the phylogenetic relationship among EBOV species (Fig. 1). On the other hand, for most of the samples from patients infected with MARV Angola, IgG antibodies to His-MARV-GP were specifically detected, except for specimen 17, which showed no IgG response to any GP. Interestingly, IgG antibodies detected in specimen 11 showed remarkable cross-reactivity with the heterologous antigens His-CIEBOV-GP and His-BEBOV-GP.

We next evaluated whether GP-specific IgM antibodies could be detected in the patient serum or plasma samples using the GP-based ELISA (Fig. 6b). The cutoff values for IgM ELISA were 0.23, 0.32, 0.31, 0.28, 0.30, 0.22, and 0.36 for His-ZEBOV-GP, His-SEBOV-GP, His-CIEBOV-GP, His-BEBOV-GP, His-REBOV-GP, His-MARV-GP, and control

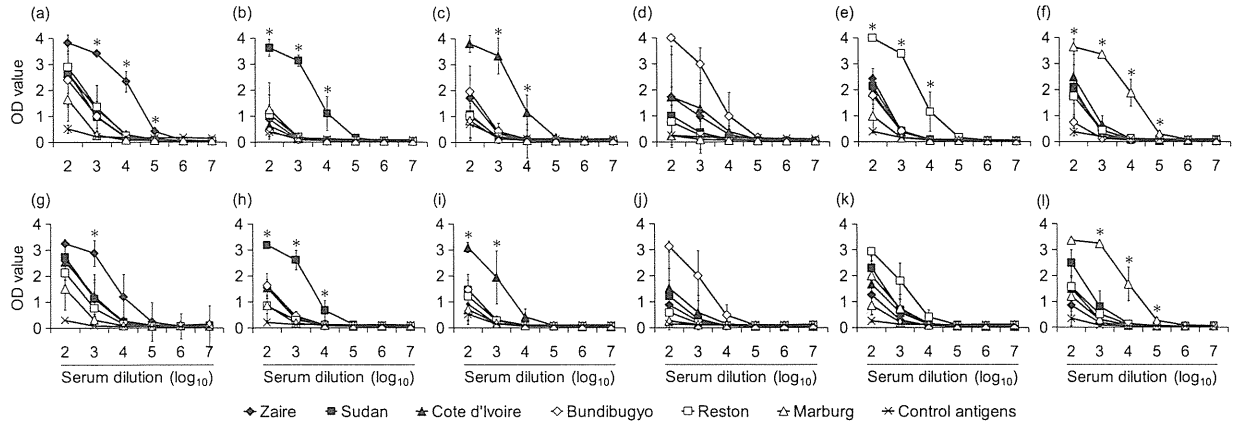

FIG. 4. IgG antibodies detected in mouse antisera. Serial 10-fold dilutions of anti-ZEBOV (a and g), anti-SEBOV (b and h), anti-CIEBOV (c and i), anti-BEBOV (d and j), anti-REBOV (e and k), and anti-MARV (f and l) sera obtained from mice immunized with EBOV and MARV VLPs were tested for IgG antibodies reacting with His-GPs (a, b, c, d, e, and f) and VLPs (g, h, i, j, k, and l). Averages and standard deviations for three mice of each group are shown. Asterisks indicate statistically significant differences in OD values between the homologous antigen and all other antigens ($P < 0.05$).

antigens, respectively. ZEBOV- or SEBOV-specific IgM antibodies were detected only in patients 2, 6, 9, and 10. In contrast, MARV-specific IgM antibodies were detected in 8 out of the 11 specimens derived from MARV Angola-infected patients. No obvious IgM cross-reactivity to heterologous GP antigens was found in these samples.

DISCUSSION

In this study, we established a GP-based ELISA to detect filovirus species-specific antibodies. To date, lysates from Vero E6 cells infected with live EBOV and MARV or recombinant EBOV and MARV NPs have been used as antigens in ELISAs for the detection of filovirus-specific antibodies (5, 7, 17). Since the NPs of EBOV and MARV contain similar amino acid sequences (18), common antibody epitopes seem to be present (12). Indeed, cross-reactivity among all EBOV species was to be expected (16, 17). Therefore, NP antigens may be useful for the detection of genus-specific antibodies but not for the detection of species-specific humoral responses (7, 16, 17).

The heterogeneity of EBOV and MARV GPs has been demonstrated at the genetic level through sequence analyses (17, 19). An ELISA using recombinant ZEBOV GP expressed in a baculovirus-insect cell expression system was reported previously (16), but it is known that the protein glycosylation pathways in insect cells differ from those in mammalian cells (6). This may significantly affect the antigenic properties of filovirus GPs, since large amounts of both N- and O-linked carbohydrate chains are present in GP molecules. To overcome this difficulty, we used mammalian 293T cells for the expression of GP antigens and verified the sensitivity and specificity of GP-based ELISAs. Our results were consistent with a previous study suggesting that anti-EBOV GP antibodies were highly species specific and showed little cross-reactivity to GPs of other EBOV species (27). These findings indicated that most antibodies induced against filovirus GPs recognized

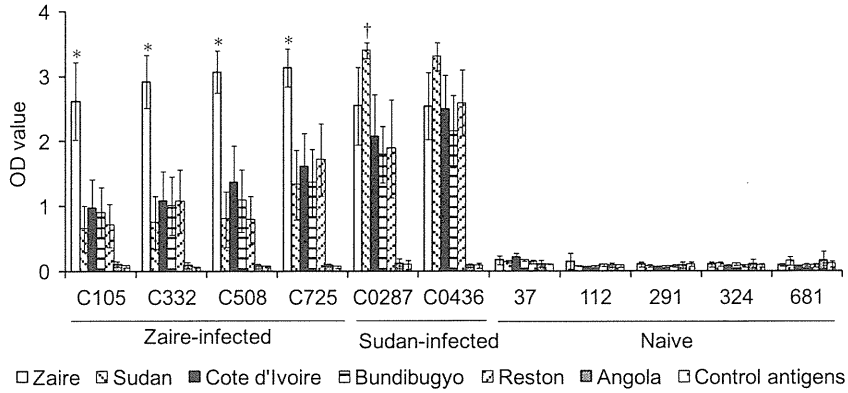

FIG. 5. IgG antibodies detected in experimentally infected monkey plasma by ELISA using His-GPs. Monkeys C105, C332, C508, and C725 were infected with ZEBOV, whereas monkeys C0287 and C0436 were infected with SEBOV. Infected monkey sera were diluted at 1:1,000. Naïve monkey sera were diluted at 1:100. Each bar represents the average and standard deviation of data from three independent experiments. Asterisks indicate statistically significant differences in OD values between the Zaire antigen and all other antigens ($P < 0.05$). The dagger shows statistically different reactions between His-SEBOV-GP and all the other antigens ($P < 0.05$) except His-ZEBOV-GP.

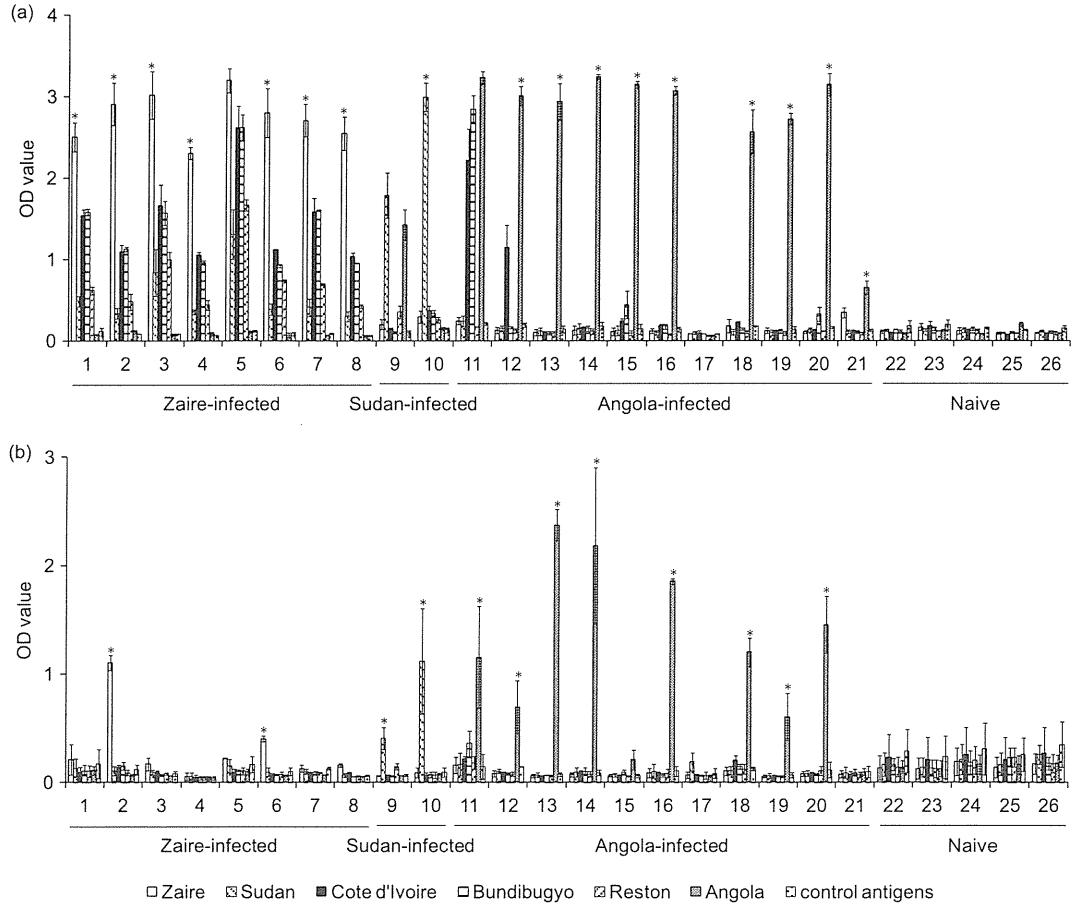

FIG. 6. IgG and IgM antibodies detected in human samples. OD values for specific IgG (a) and IgM (b) antibodies in the patient sera are shown. Sera from 21 individuals were analyzed at 1:1,000 dilutions. Naïve human sera (1:100 dilution) were used as a negative control. Each bar represents the average and standard deviation of data from independent experiments. Asterisks indicate statistically significant differences in OD values between the homologous antigen and all other antigens ($P < 0.05$).

epitopes in the variable regions of the protein. Expectedly, the serological classification mirrors the phylogenetic relationship of the different GPs (Fig. 1). Interestingly, serological characterization of anti-BEBOV antibodies clearly supports the molecular investigations (31) suggesting that BEBOV represents a new species within the EBOV genus.

IgG antibodies in some of the serum and plasma samples collected from infected monkeys and humans showed appreciable cross-reactivity to heterologous antigens, whereas antibodies in the mouse sera produced by immunization with VLPs specifically reacted to the homologous antigens. This result led us to the conjecture that VLP immunization and live-virus infection induce a distinct antibody repertoire or that the antibody repertoire of mice differs from that of primates. Interestingly, the plasma of patient 11 infected with MARV Angola contained IgG, but not IgM, antibodies cross-reactive to His-CIEBOV-GP and His-BEBOV-GP. It might be possible that prior to infection with MARV Angola, this patient was infected with CIEBOV, BEBOV, or another unknown filovirus whose GP has epitopes shared among CIEBOV and BEBOV. In the plasma of patient 17, neither IgG nor IgM antibodies were readily detected. An explanation for this observation might be differences of immunological conditions in individuals, or alternatively, the blood samples have been collected before a detectable antibody response was induced.

Notably, our GP-based ELISA detected MARV Angola-specific IgM antibodies in most of the plasma samples collected during the acute or subacute phase of infection, although it was reported previously that the detection of antibodies is of only limited use for acute-case diagnosis due to a lack of a detectable antibody response (8). The present study suggests that if proper antigen and sensitive assays are available, IgM antibodies can be useful for the diagnosis of acute EBOV and MARV infections and support the use of antigen capture ELISA and reverse transcription-PCR, the most commonly used technologies.

Despite the more recent discovery of REBOV in domestic pigs in the Philippines (1) and the discovery of fruit bat species as potential reservoirs for EBOV and MARV (9, 15, 30, 32), the search for the reservoirs and potential amplifying hosts remains ongoing. Advanced diagnostic technologies are welcome here, and our new GP-based species-specific antibody detection ELISA may be a useful tool for future ecological and seroepidemiological studies in areas of Central Africa and parts of Asia where the disease is endemic.

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Journal of Bacteriology

Complete Genome Sequence of Mycoplasma pneumoniae Type 2a Strain 309, Isolated in Japan

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GENOME ANNOUNCEMENT

Complete Genome Sequence of *Mycoplasma pneumoniae* Type 2a Strain 309, Isolated in Japan

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***Mycoplasma pneumoniae* strain 309, a type 2a (subtype 2 variant) strain of this bacterium, has variations in the P1 protein, which is responsible for attachment of the bacterium to host cells. Here, we report the complete genome sequence of *M. pneumoniae* strain 309 isolated from a pneumonia patient in Japan.**

Mycoplasma pneumoniae is a common pathogen that causes atypical pneumonia and bronchitis in humans, particularly in children and young adults (1, 11). Clinical isolates of this bacterium can be classified into two major groups (subtypes 1 and 2) based on nucleotide sequence variations in the *p1* gene, which encodes an essential factor responsible for cytadherence and pathogenesis (6, 7, 9, 10). Here, we report the complete genome sequence of *M. pneumoniae* strain 309, one of the first-discovered type 2a strains, which was isolated in Hokkaido, Japan, in 1998 (6).

The genome was sequenced using a Roche 454 GS Junior sequencer. A single analysis generated 77.2-Mb sequences (151,617 reads; average length, 509 bp), providing approximately 95-fold genome coverage. Sequences were assembled using GS *de novo* assembler v. 2.5p1; 8 contigs, from 416 to 0.5 kb in size, resulted. We combined these contigs into a circular genome using Sanger sequencing of PCR amplicons derived using primers specific to contig termini. During the genome annotation process, we identified 84 suspected sequencing errors caused by 454 pyrosequencing. These sites were resequenced by Sanger sequencing; 23 pyrosequencing errors were confirmed and corrected.

The complete genome of *M. pneumoniae* strain 309 encompasses 817,176 bp of chromosomal DNA (39.98% GC content), containing 707 predicted coding sequences (CDS), 1 rRNA operon, 36 tRNAs, and 4 noncoding RNA genes. This genome is most similar to those of *M. pneumoniae* M129 and FH (GenBank accession numbers NC000912 and CP002077, respectively), subtype 1 and 2 strains, respectively (2, 8).

A notable difference between these genomes and that of strain 309 is a 6-kb insertion at MPNA5870 in strain 309; this position corresponds to MPN586 of the M129 genome. The M129 and FH genomes are nearly identical at this position. MPNA5870 (MPN586) and several neighboring CDS are putative lipoprotein-encoding genes that are similar to each other but not identical. The 6-kb insertion in the strain 309 genome contains five additional putative lipoprotein genes. Unexpectedly, comparison of the M129, FH, and 309 strains from our laboratory by PCR revealed that strain FH also included an approximately 5-kb insertion at this position. Whether the FH in our laboratory and the genome-sequenced FH strains are in fact different is unclear. However, it is likely that *M. pneumoniae* strains vary in this region, involving a change in the number of putative lipoprotein genes.

Type 2a strains were rarely detected in the 1990s, but, after 2003, they have frequently been found in clinical specimens in Japan (5), consistent with reports from other countries (3, 4, 12). Precise comparisons of strain 309 and other *M. pneumoniae* genomes will identify differences that affect surface molecules, such as lipoproteins or cytadherence proteins, thereby changing their antigenicity. Such information is crucial for understanding the recent increase of type 2a strains. The genome sequence reported here may also be useful in developing strategies for treatment of *M. pneumoniae* infections.

Nucleotide sequence accession number. The sequence data for *M. pneumoniae* strain 309 have been deposited in DDBJ/EMBL/GenBank databases under accession number AP012303.

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Disinfection Methods for Spores of *Bacillus atrophaeus*, *B. anthracis*, *Clostridium tetani*, *C. botulinum* and *C. difficile*

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To evaluate disinfection methods for environments contaminated with bioterrorism-associated microorganism (*Bacillus anthracis*), we performed the following experiments. First, the sporicidal effects of sodium hypochlorite on spores of five bacterial species were evaluated. *Bacillus atrophaeus* was the most resistant to hypochlorite, followed in order by *B. anthracis*, *Clostridium botulinum* and *Clostridium tetani*, and *Clostridium difficile*. Subsequently, using *B. atrophaeus* spores that were the most resistant to hypochlorite, the sporicidal effects of hypochlorite at lower pH by adding vinegar were evaluated. Hypochlorite containing vinegar had far more marked sporicidal effects than hypochlorite alone. Cleaning with 0.5% (5000 ppm) hypochlorite containing vinegar inactivated *B. atrophaeus* spores attached to vinyl chloride and plywood plates within 15 s, while that not containing vinegar did not inactivate spores attached to cement or plywood plates even after 1 h. Therefore, the surfaces of cement or plywood plates were covered with gauze soaked in 0.5% hypochlorite containing vinegar, and the sporicidal effects were evaluated. *B. atrophaeus* spores attached to plywood plates were not inactivated even after 6 h, but those attached to cement plates were inactivated within 5 min. On the other hand, covering the surfaces of plywood plates with gauze soaked in 0.3% peracetic acid and gauze soaked in 2% glutaral inactivated *B. atrophaeus* spores within 5 min and 6 h, respectively. These results suggest that hypochlorite containing vinegar is effective for disinfecting vinyl chloride, tile, and cement plates contaminated with *B. anthracis*, and peracetic acid is effective for disinfecting plywood plates contaminated with such microorganism.

Key words bioterrorism; *Bacillus anthracis*; disinfection; sodium hypochlorite; vinegar

The forms of microorganism that are the most resistant to disinfectants are spores. However, data on species that are most resistant to disinfectants among spore-forming bacteria such as *Bacillus atrophaeus*, *Bacillus anthracis*, *Clostridium tetani*, *Clostridium botulinum*, and *Clostridium difficile* remain incomplete.[1,2] Therefore, we first evaluated the effects of sodium hypochlorite as a multipurpose disinfectant with sporicidal effects on the spores of these species.[3—6] Subsequently, to evaluate disinfection methods for environments contaminated with *B. anthracis*, we used *B. atrophaeus* spores that showed the highest resistance to the disinfectant as a substitute microorganism and evaluated disinfection methods for environments contaminated with *B. atrophaeus* spores.

MATERIALS AND METHODS

Agents and Strains Used The disinfectant products used were the 6% sodium hypochlorite Yoshida (pH 11.7, Yoshida Pharmaceutical Co., Tokyo, Japan), 3% peracetic acid (Aceside®, Saraya Co., Osaka, Japan), and 2% glutaral (Sterihyde®, Maruishi Pharmaceutical Co., Osaka, Japan) solutions. Gereal vinegar (*ca.* 3.9% acetic acid; Mizkan Co., Aichi, Japan) was used as the vinegar. In addition, nutrient broth (Eiken Chemical Co., Tokyo, Japan) containing 0.5% sodium thiosulfate (Katayama Chemical Co., Tokyo, Japan) was used as an inactivator of sodium hypochlorite and peracetic acid, and 5% glycine solution (Kanto Chemical Co., Tokyo, Japan) as an inactivator of glutaral.[7—9]

Five bacterial strains were evaluated: *B. atrophaeus* ATCC6633, *B. anthracis* Pasteur II, *C. tetani* KZ1113, *C. botulinum* type A 62A, and *C. difficile* ACTT9689. For spore preparation of *B. atrophaeus*, fresh cultures grown in nutrient broth were spread on nutrient agar (Eiken Chemical Co., Japan), and incubated at 37 °C for 4 d. After 4 d, the spores were collected and washed several times with physiological saline using the centrifugal sedimentation technique. To kill the vegetative cells, the suspension was heated at 80 °C for 10 min. A spore suspension containing 10^8 colony forming units (cfu)/ml was obtained.

For spore preparation of *B. anthracis*, fresh cultures grown in Luria–Bertani broth were spread on a low-nutrient agar [0.8% nutrient broth and 1% yeast extract (Difco, Detroit, U.S.A.), 0.05% $MgSO_4 \cdot 7H_2O$, 0.2% KCl, 1.6% agar, 0.1% $MnSO_4$, 0.1% glucose, 0.1% $Ca(NO_3)_2$, and 0.1% $FeSO_4$], and incubated at 32 °C for 7 d. After 7 d, the spores were collected and washed 10 times with chilled sterile distilled water. To kill the vegetative cells, the suspension was incubated at 85 °C for 20 min. The suspension was then washed several times with distilled water, and the spore suspension containing 10^8 cfu/ml obtained.

For spore preparation of *C. tetani*, a strain was anaerobically cultured (Anaeropack; Mitsubishi Gas Chemical Company Inc., Tokyo, Japan) in Takahashi medium (30 g of proteose peptone, 10 g of meat extract, 2 g of NaCl, 20 g of bacto agar, and 1000 ml of distilled water, pH 7.4) at 37 °C for 7 d, and spores were scraped off using a platinum loop, suspended in physiological saline, washed several times with physiological saline using the centrifugal sedimentation technique. To kill the vegetative cells, the suspension heated at

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80 °C for 10 min. A Spore suspension to yield a spore preparation containing 10^7 cfu/ml was abtained.

For spore preparation of *C. botulinum*, a strain was anaerobically cultured in TP medium (5 g of trypticase peptone, 0.5 g of bacto peptone, 0.1 g of sodium mercaptoacetate, and 100 ml of distilled water) at 30 °C for 7 d. The suspension was then washed several times with distilled water, using the centrifugal sedimentation technique. To kill the vegetative cells, the suspension was heated at 60 °C for 15 min. A spore sustension containing 10^6 cfu/mL was abtained.

For spore preparation of *C. difficile*, a strain was anaerobically cultured in mBHI agar medium at 37 °C for 5 d. Spores were scraped off using a platinum loop, suspended in physiological saline, washed several times with physiological saline employing the centrifugal sedimentation technique. To kill the vegetative cells, the suspension was heated at 70 °C for 10 min to yield a spore preparation containing 10^7 cfu/ml was abtained.

Sporicidal Effects of Sodium Hypochlorite on Spores of 5 Bacterial Species in Suspension Tests The sporicidal effects of 0.1% (1000 ppm) sodium hypochlorite on the spores of *B. atrophaeus* and *B. anthracis* were evaluated employing the following procedure at room temperature of 22±2 °C. After the addition of 0.1 ml of spore preparations to 0.9 ml of 0.1% sodium hypochlorite, the mixture was stirred for 5 s, and 0.1 ml was collected at each time point and immediately added to 0.9 ml of nutrient broth containing 0.5% sodium thiosulfate, which was stirred for 5 s and left to stand for 10 min. The suspensions were serially diluted 10-fold with sterile saline. The undiluted or diluted samples (0.2 ml) were plated on nutrient agar and incubated 37 °C for 48 h, after which the viable spores were counted.

The sporicidal effects of sodium hypochlorite on the spores of *C. tetani*, *C. botulinum*, and *C. difficile* were evaluated employing the following procedure at room temperature of 22±2 °C. After the addition of 0.1 ml of spore preparations to 0.9 ml of 0.1% sodium hypochlorite, the mixture was stirred for 5 s, and 0.1 ml was collected at each time point and immediately added to 0.9 ml of nutrient broth containing 0.5% sodium thiosulfate, which was stirred for 5 s and left to stand for 10 min. The suspensions were serially diluted 10-fold with sterile saline. The undiluted or diluted samples (0.2 ml) were plated on modified GAM agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and incubated anaerobically at 37 °C for 96 h for *C. tetani*, on GAM agar (Nissui Pharmaceutical Co., Ltd.) at 37 °C for 72 h for *C. botulinum*, and on CCMA agar (Nikken Bio Medical Laboratory Co., Ltd., Tokyo, Japan) at 37 °C for 72 h for *C. difficile*.

Sporicidal Effects of Sodium Hypochlorite Alone and Sodium Hypochlorite Containing Vinegar on *B. atrophaeus* Spores in Suspension Tests The sporicidal effects of 0.1% or 0.5% sodium hypochlorite, a mixture of 5% (50000 ppm) sodium hypochlorite, water, and vinegar in a ratio of 1 : 8 : 1 (0.5% sodium hypochlorite containing vinegar), and a mixture of 1% sodium hypochlorite, water, and vinegar in a ratio of 1 : 8 : 1 (0.1% sodium hypochlorite containing vinegar) were evaluated using the following procedure at room temperature of 22±2 °C. After the addition of 0.1 ml of spore preparation to 0.9 ml of sodium hypochlorite or sodium hypochlorite containing vinegar, the mixture was stirred for 5 s, and 0.1 ml was collected at each time point of analysis and immediately added to 0.9 ml of nutrient broth containing 0.5% sodium thiosulfate, which was stirred for 5 s and left to stand for 10 min. The suspensions were serially diluted 10-fold with sterile saline. The undiluted and diluted samples (0.2 ml) were plated on nutrient agar at 37 °C for 48 h.

Sporicidal Effects of Disinfectants on *B. atrophaeus* Spores Attached to Environmental Surfaces After 0.1 ml of the spore suspension of *B. atrophaeus* ATTC6633 (1.0×10^8/ml) was placed on the carrier surface (vinyl chloride 2×3 cm$=6$ cm^2, tile 6 cm^2, cement 6 cm^2, and plywood 6 cm^2 plates), it was allowed to air-dry for 2 h in a class 2 biological safety cabinet. Subsequently, the plates were wiped with cotton swabs soaked in sodium hypochlorite or sodium hypochlorite containing vinegar so that the entire surface was wet. These plates were immersed in 20 ml of nutrient broth containing 0.5% sodium thiosulfate at each time point of analysis and ultrasonicated at 37 kHz for 10 min before the spores were counted.[10] Experiments using vinyl chloride plates were performed both at room temperature of 22±2 °C and in an incubator at 10 °C.

For cement and plywood plates, another disinfection method was also evaluated. These plates were covered with 2 sheets of sterile gauze (100% plain-weave cotton gauze; Kawamoto Sangyo Co., Ltd., Osaka, Japan: 2×3 cm) soaked in 0.8 ml of 0.5% sodium hypochlorite, sodium hypochlorite containing vinegar, 0.3% peracetic acid, or 2% glutaral. At each time point of analysis, plates treated with sodium hypochlorite, sodium hypochlorite containing vinegar, or peracetic acid were immersed in 20 ml of nutrient broth containing 0.5% sodium thiosulfate and those treated with glutaral in 20 ml of 5% glycine solution and ultrasonicated at 37 kHz for 10 min before the spores were counted. This experiment was carried out at room temperature of 22±2 °C.

Stability of Sodium Hypochlorite Containing Vinegar The residual chlorine concentration in sodium hypochlorite after the addition of vinegar was determined at each time point of analysis at room temperature of 22±2 °C. Available chlorine was measured using a pocket colorimeter 46700-00 (Central Kagaku Corp., Tokyo, Japan). The pH of sodium hypochlorite or sodium hypochlorite containing vinegar was measured using a pH meter F-7 type (Horiba Inc., Kyoto, Japan). Experiments were performed twice, and the mean value was calculated.

RESULTS

Table 1 shows the effect of 0.1% (1000 ppm) sodium hypochlorite on the spores of the 5 bacterial species in suspension tests. This disinfectant inactivated the spores of *B. atrophaeus* within 30 min, those of *B. anthracis* within 20 min, those of *C. botulinum* and *C. tetani* within 5 min, and those of *C. difficile* within 1 min.

Table 2 shows the sporicidal effects of sodium hypochlorite or sodium hypochlorite containing vinegar on *B. atrophaeus* spores in suspension tests. *B. atrophaeus* spores were inactivated within 30 min and 20 min by 0.1% and 0.5% sodium hypochlorite and within 30 s and 15 s by 0.1% and 0.5% sodium hypochlorite containing vinegar, respectively.

Table 3 shows the sporicidal effects of wiping with sodium hypochlorite or sodium hypochlorite containing vinegar on

Table 1. Sporicidal Effects of 0.1% (1000 ppm) Sodium Hypochlorite on Spores of Five Bacterial Species in Suspension Tests (22±2 °C)

Bacterial species \ Contact time	Spore count (cfu/ml) at							
	0	1 min	5 min	10 min	20 min	30 min	1 h	2 h
Bacillus atrophaeus	1.1×10^7	1.0×10^7	6.3×10^6	7.5×10^6	1.7×10^6	<50	<50	<50
Bacillus anthracis	1.1×10^7	1.3×10^6	7.0×10^5	1.3×10^3	<50	<50	<50	<50
Clostridium botulinum	2.0×10^5	9.0×10^4	<50	<50	<50	<50	<50	<50
Clostridium tetani	3.9×10^6	4.9×10^4	<50	<50	<50	<50	<50	<50
Clostridium difficile	5.0×10^5	<50	<50	<50	<50	<50	<50	<50

Table 2. Sporicidal Effects of Sodium Hypochlorite and Sodium Hypochlorite Containing Vinegar on *Bacillus atrophaeus* Spores in Suspension Tests (22±2 °C)

Agent \ Contact time	Spore count (cfu/ml) at								
	0	15 s	30 s	1 min	5 min	10 min	20 min	30 min	60 min
0.1% Sodium hypochlorite	1.1×10^7	NT[a)]	NT	1.0×10^7	6.3×10^6	7.5×10^6	1.7×10^6	<50	<50
0.1% Sodium hypochlorite containing vinegar	1.4×10^7	75	<50	<50	<50	<50	<50	<50	<50
0.5% Sodium hypochlorite	1.5×10^7	NT	NT	1.3×10^7	7.8×10^6	1.0×10^6	<50	<50	<50
0.5% Sodium hypochlorite containing vinegar	1.0×10^7	<50	<50	<50	<50	<50	<50	<50	<50
10-Fold dilution of vinegar	7.5×10^6	NT	NT	NT	NT	6.5×10^6	8.0×10^6	6.5×10^6	7.0×10^6

[a)] NT, not tested.

Table 3. Sporicidal Effects of Wiping with Sodium Hypochlorite and with Sodium Hypochlorite Containing Vinegar on Spores Attached to Various Types of Plate (22±2 °C)

Plate	Agent \ Contact time	Spore count (cfu/ml) at								
		0	15 s	30 s	1 min	5 min	10 min	20 min	30 min	60 min
Vinyl chloride	0.1% Sodium hypochlorite	2.4×10^6	NT[a)]	NT	2.7×10^3	3.7×10^3	2.8×10^3	1.7×10^3	<100	<100
	0.1% Sodium hypochlorite containing vinegar	1.9×10^6	4.0×10^4	5.3×10^3	2.7×10^3	1.8×10^3	1.9×10^3	<100	<100	<100
	0.5% Sodium hypochlorite	2.1×10^7	NT	NT	9.0×10^4	4.0×10^5	3.7×10^2	<100	<100	<100
	0.5% Sodium hypochlorite containing vinegar	5.5×10^6	<100	<100	<100	<100	<100	<100	<100	<100
Tile	0.1% Sodium hypochlorite	8.0×10^6	NT	NT	6.0×10^4	1.1×10^4	1.0×10^4	2.1×10^4	2.4×10^5	1.0×10^5
	0.1% Sodium hypochlorite containing vinegar	4.1×10^6	NT	NT	2.0×10^4	3.5×10^4	1.5×10^2	2.5×10^2	1.5×10^3	<100
	0.5% Sodium hypochlorite	1.1×10^6	NT	NT	2.2×10^3	5.8×10^2	2.1×10^3	<100	<100	<100
	0.5% Sodium hypochlorite containing vinegar	1.9×10^6	<100	<100	<100	<100	NT	NT	NT	NT
Cement	0.1% Sodium hypochlorite	1.6×10^7	NT	NT	NT	NT	1.8×10^6	2.0×10^6	2.6×10^6	2.0×10^6
	0.1% Sodium hypochlorite containing vinegar	3.1×10^6	NT	NT	NT	NT	1.8×10^6	2.0×10^6	1.9×10^6	1.6×10^6
	0.5% Sodium hypochlorite	1.7×10^6	NT	NT	NT	NT	1.0×10^6	4.0×10^4	1.4×10^6	1.1×10^6
	0.5% Sodium hypochlorite containing vinegar	1.3×10^7	NT	NT	NT	NT	2.4×10^6	1.1×10^6	8.8×10^6	3.8×10^6
Plywood	0.1% Sodium hypochlorite	1.5×10^6	NT	NT	NT	NT	3.4×10^6	2.2×10^6	2.1×10^6	1.1×10^6
	0.1% Sodium hypochlorite containing vinegar	2.2×10^6	NT	NT	NT	NT	1.6×10^6	1.5×10^6	1.5×10^6	6.5×10^5
	0.5% Sodium hypochlorite	1.7×10^6	NT	NT	NT	NT	1.4×10^6	1.2×10^6	8.4×10^5	1.4×10^6
	0.5% Sodium hypochlorite containing vinegar	2.5×10^6	NT	NT	NT	NT	3.0×10^5	2.0×10^5	3.0×10^5	2.4×10^5

[a)] NT, not tested.

B. atrophaeus spores attached to various types of plate (vinyl chloride, tile, cement, and plywood). *B. atrophaeus* spores attached to vinyl chloride or tile plates were inactivated within 20 min after wiping with 0.1% sodium hypochlorite containing vinegar and within 15 s after wiping with 0.5% sodium hypochlorite containing vinegar. However, *B. atrophaeus* spores attached to cement or plywood plates were not inactivated even 1 h after wiping with 0.5% sodium hypochlorite containing vinegar. Table 4 shows the sporicidal effects of wiping with 0.5% sodium hypochlorite and sodium hypochlorite containing vinegar on *B. atrophaeus* spores attached to vinyl chloride plates at a temperature of 10 °C. This experiment at 10 °C was done to know the efficacy of these disinfectants in a cool place. Compared with 22±2 °C, the time required for inactivation was slightly prolonged, but spores were inactivated within 1 min after wiping with 0.5% sodium hypochlorite containing vinegar.

Table 5 shows the sporicidal effects of covering with gauze soaked in 0.5% sodium hypochlorite and sodium hypochlorite containing vinegar on *B. atrophaeus* spores attached to cement or plywood plates. *B. atrophaeus* spores attached to cement plates were inactivated within 5 min, but those attached to plywood plates were not inactivated even 6 h after covering with gauze soaked in 0.5% sodium hypochlorite containing vinegar.

Table 6 shows the sporicidal effects of covering with gauze

Table 4. Sporicidal Effects of Wiping with 0.5% (5000 ppm) Sodium Hypochlorite Containing Vinegar on *Bacillus atrophaeus* Spores Attached to Vinyl Chloride Plates (10 °C)

Agent \ Contact time	Spore count (cfu/plate) at								
	0	15 s	30 s	1 min	5 min	10 min	20 min	30 min	60 min
0.5% Sodium hypochlorite	6.1×10^6	NT[a)]	NT	NT	2.0×10^5	8.5×10^4	2.5×10^4	5.0×10^3	<100
10-Fold dilution of vinegar	6.1×10^6	NT	NT	NT	NT	NT	NT	5.2×10^6	5.2×10^6
0.5% Sodium hypochlorite containing vinegar	6.1×10^6	8.5×10^2	6.0×10^2	<100	<100	<100	<100	<100	<100

a) NT, not tested.

Table 5. Sporicidal Effects of Covering Cement or Plywood Plates with Gauze Soaked in 0.5% (5000 ppm) Sodium Hypochlorite Containing Vinegar (22±2 °C)

Plate	Agent \ Contact time	Spore count (cfu/plate) at										
		0	15 s	30 s	1 min	5 min	10 min	30 min	1 h	2 h	3 h	6 h
Cement	0.5% Sodium hypochlorite	3.3×10^6	9.6×10^5	8.2×10^5	4.0×10^5	<100	<100	<100	NT[a)]	NT	NT	NT
	0.5% Sodium hypochlorite containing vinegar	2.0×10^6	9.0×10^5	1.7×10^5	6.0×10^2	<100	<100	<100	NT	NT	NT	NT
Plywood	0.5% Sodium hypochlorite	1.9×10^6	NT	NT	NT	NT	NT	NT	7.3×10^4	3.8×10^5	2.2×10^4	1.7×10^5
	0.5% Sodium hypochlorite containing vinegar	1.4×10^6	NT	NT	NT	NT	NT	NT	8.2×10^3	6.4×10^3	1.3×10^4	2.5×10^4

a) NT, not tested.

Table 6. Sporicidal Effects of Covering Plywood Plates with Gauze Soaked in Disinfectants on *Bacillus atrophaeus* Spores Attached to the Plates (22±2 °C)

Agent \ Contact time	Spore count (cfu/plate) at										
	0	15 s	30 s	1 min	5 min	10 min	30 min	1 h	2 h	3 h	6 h
1% Sodium hypochlorite	1.0×10^6	NT[a)]	NT	NT	NT	NT	NT	1.6×10^5	5.8×10^3	6.5×10^4	4.4×10^3
5% Sodium hypochlorite	1.0×10^6	NT	NT	NT	NT	NT	NT	1.5×10^3	1.6×10^3	1.5×10^2	3.5×10^2
0.3% Peracetic acid	1.0×10^6	2.2×10^6	5.8×10^4	3.0×10^2	<100	<100	<100	<100	NT	NT	NT
2% Glutaral	1.5×10^6	NT	NT	NT	NT	NT	NT	9.5×10^3	7.0×10^3	1.5×10^2	<100

a) NT, not tested.

Table 7. Stability of Sodium Hypochlorite Containing Vinegar

Agent	pH	% Remaining of free available chlorine at			
		Immediately after	10 min	1 h	24 h
0.01% Sodium hypochlorite containing vinegar	2.89	14	4	3	1
0.01% Sodium hypochlorite	9.97	100	103	101	96
0.1% Sodium hypochlorite containing vinegar	3.97	72	63	54	18
0.1% Sodium hypochlorite	10.25	98	100	99	95
0.5% Sodium hypochlorite containing vinegar	5.65	96	90	81	48
0.5% Sodium hypochlorite	11.35	99	100	98	103

soaked in 1% (10000 ppm) or 5% (50000 ppm) sodium hypochlorite, 0.3% peracetic acid, or 2% glutaral on *B. atrophaeus* spores attached to plywood plates. *B. atrophaeus* spores attached to plywood plates were not inactivated by 1% or 5% sodium hypochlorite even after 6 h but inactivated within 5 min by 0.3% peracetic acid and within 6 h by 2% glutaral.

Table 7 shows the pH of sodium hypochlorite and sodium hypochlorite containing vinegar, and changes in the stability of sodium hypochlorite after the addition of vinegar (temperature, 22±2 °C). The percentage of remaining free available chlorine in sodium hypochlorite 24 h after the addition of vinegar was 1% in 0.01% solution, 18% in 0.1% solution, and 48% in 0.5% solution. Thus, after the addition of vinegar, the residual chlorine concentration in sodium hypochlorite decreased rapidly.

DISCUSSION

The present experiments revealed that *B. atrophaeus* is the most resistant, followed in order by *B. atrophaeus*, *B. anthracis*, *C. tetani* and *C. botulinum*, and *C. difficile* among the spores of the five bacterial species examined. On-one hand, Bacterial spores are more difficult to kill than other

micro-organisms by disinfectants. Thus, if the spores of *B. atrophaeus* can be killed, all bioterrorism-associated microorganisms including *B. anthracis* should be killed.[2,11]

Therefore, assuming environmental contamination with *B. anthracis*, we performed experiments using the spores of *B. atrophaeus* as a substitute microorganism. Evaluation of the sporicidal effects of wiping with sodium hypochlorite and sodium hypochlorite containing vinegar on *B. atrophaeus* spores showed rapid sporicidal effects of 0.5% sodium hypochlorite containing vinegar on spores attached to vinyl chloride and tile plates. This rapid effect was also observed at 10 °C. Sodium hypochlorite with a decreased pH due to the addition of vinegar has been shown to exhibit more marked sporicidal effects than sodium hypochlorite alone,[3,12] and similar results were obtained in this study. However, even sodium hypochlorite containing vinegar did not have sporicidal effects on spores attached to cement or plywood plates. Therefore, to kill *B. atrophaeus* spores attached to cement or plywood plates, the plates were covered with gauze soaked in 0.5% sodium hypochlorite or sodium hypochlorite containing vinegar. Good sporicidal effects on *B. atrophaeus* spores attached to cement plates were observed. However, even covering with gauze soaked in 0.5% sodium hypochlorite containing vinegar could not kill *B. atrophaeus* spores attached to plywood plates. This may be due to inactivation of this agent by wood.[13,14] In addition, to inactivate *B. atrophaeus* spores attached to plywood plates, the plates were covered with gauze soaked in sodium hypochlorite at high concentrations (1% and 5%), 0.3% peracetic acid, or 2% glutaral. The spores were inactivated by 0.3% peracetic acid and 2% glutaral within 5 min and 6 h, respectively. Peracetic acid and glutaral are highly toxic due to irritation of the respiratory tract and eye mucosae by their vapors and therefore should not be used for environmental disinfection.[15,16] However, since sodium hypochlorite is ineffective in inactivating spores attached to wooden materials such as plywood, peracetic acid should be used with care taken to prevent human exposure to its vapors.

These results suggest the usefulness of 0.5% sodium hypochlorite containing vinegar for disinfecting environmental surfaces, excluding wooden materials such as plywood, contaminated with bioterrorism-associated microorganisms. In addition, peracetic acid may be useful for disinfecting wooden materials such as plywood. However, the residual chlorine concentration decreased by about half 24 h after the addition of vinegar to sodium hypochlorite. Therefore, sodium hypochlorite containing vinegar should be prepared immediately before use, and used also with care taken to prevent exposure to its chlorine gas.

Acknowledgement This work was supported by Grants (H20-shinkou-Ippan-006) from the Health and Labour Sciences Research Grants, Japan.

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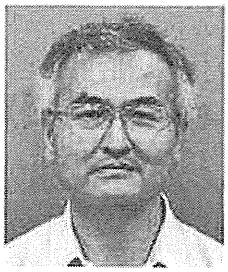
内部被曝とその考え方

Internal contamination with radionuclide



明石真言
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放射線医学総合研究所理事

◎“平成23年(2011年)東北地方太平洋沖地震”に伴う原子力発電所事故が発生してから7カ月以上の月日が流れた．いまだ事故が終息したわけではないが，国民は落ち着きを取り戻しはじめてきた一方，不安は環境からの外部被曝のみならず，とくに食品由来のものも含めた内部被曝にまで生じている．残念なことに内部被曝には誤解が多く，症状や線量とその評価について，医療従事者であっても正しい理解がされていない．内部被曝を評価する際に使う体外計測計(ホールボディカウンタ；whole body counter：WBC)からは測定時の体内の放射性物質の量を知ることはできるが，内部被曝を線量シーベルト(Sv)で表すためには摂取時期や経路を考慮し別途計算が必要となる．線量も預託実効線量という，外部被曝線量とは異なった概念で示すためわかりにくい．当然体内に摂取された放射性物質は安定型の同位体と同じ代謝が行われるため，体内被曝の線量評価には放射線の物理学的ばかりでなく生物学的に代謝などの動態や性質を考慮し，複雑な計算が行われる．このようにWBC測定から内部被曝線量を出すためにはいくつかの過程が必要である．内部被曝を正しく理解し評価するために必要な基礎項目を概説する．

Key word：内部被曝，ホールボディカウンタ(WBC)，預託実効線量，線量評価

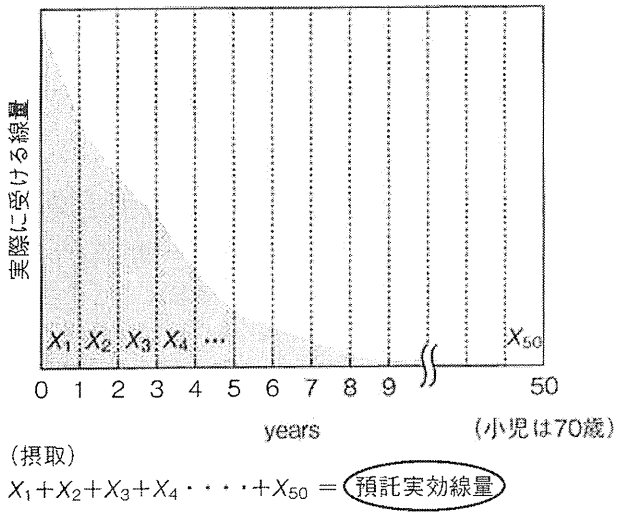
自然界には，放射線および放射性物質が存在する．自然起源の放射性核種を含む物質(放射性物質という)を自然起源放射性物質(naturally occurring radioactive materials：NORM)といい，起源の違いによって「地球形成過程で宇宙空間から地球に取り込まれた放射性核種」と「宇宙線によって自然に生成される放射性核種」に分けられる．地球形成過程で宇宙空間から地球に取り込まれた代表的な自然起源の放射性核種には，K-40，Rb-87(ルビジウム)，Pb-210，Po-210(ポロニウム)などがあり，宇宙線によって自然に生成されるおもな自然起源の放射性核種にはC-14がある[1]．これらは土壌，岩石中にあるため，食品を通じて体内にも摂取され，体内放射性物質になる．体重60 kgの標準的日本人男子の体内放射性物質は平均4,000 BqのK-40と2,500 BqのC-14である(表1)[2]．体内の放射性物質から受ける被曝は1年で約0.3 mSvとされており，内部被曝線量のひとつの目安となる．表2に食品中に含まれるK-40の量も示す[3]．

内部被曝の線量評価の考え方

放射性物質が体内に摂取されると，この放射性物質が体内に存在し放射線を出しているかぎり内部被曝が起こる．放射性物質は時間とともに原子数が減少し，放射線を出す能力が減少する．これを物理学的半減期という．一方，体内に吸収された放射性物質はその性質に従い代謝され，体外へと排出される．体内に吸収された物質量が半分になるまでの時間を示すのが生物学的半減期という．つまり物理学的に放射線を出している時間と，体内にとどまっている時間の両者を考えることで，線量を評価する．この物理学的半減期と生物学的半減期の両者を考慮した半減期を実効半減期といい，この考え方に基づいて線量を計算することになる．物理学的半減期が同じであれば，生

表 1　体内に存在する放射性核種(体重60kgの日本人の場合)[2]

核種	放射能(Bq)
カリウム 40(K-40)	4,000
炭素 14(C-14)	2,500
ルビジウム 87	500
鉛 210・ポロニウム 210	20



(摂取)

$X_1+X_2+X_3+X_4\cdots\cdots+X_{50}=$ 預託実効線量

図 1　預託実効線量

内部被曝は預託実効線量として表し，成人ならば今後 50 年間，小児の場合は 70 歳までに被曝する積算量をいう．

表 2　食品中のカリウム40の放射能[3]

食品名	放射能(Bq/kg)
干し昆布	2,000
干ししいたけ	700
生わかめ	200
ほうれん草	200
魚	100
牛肉	100
牛乳	50
米	30
食パン	30
ポテトチップ	400
ビール	10

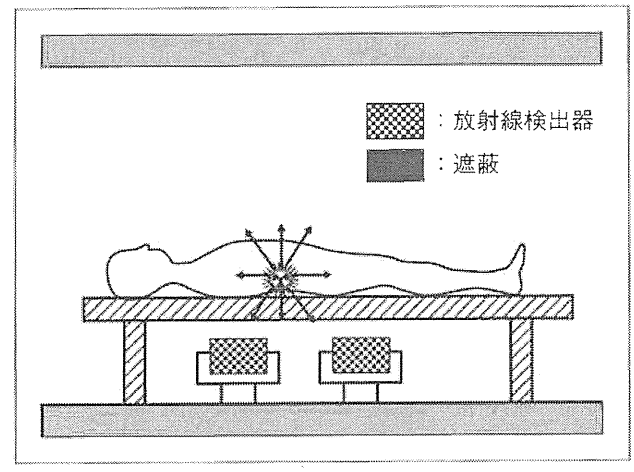


図 2　ベッド型ホールボディカウンタ(WBC)

環境放射能の影響を減らすための遮蔽は検出器のまわりだけに限られている．このような簡易な遮蔽は被検者のアクセスのしやすさや心理的な圧迫感を減らすためである．ベッド型のほかに椅子型，立位型の WBC もある．

物学的半減期が短いほど被曝線量は小さくなる．また，逆に，生物学的半減期が同じであれば，物理学的半減期が長いほど被曝線量は大きい，ということになる．

もうひとつ内部被曝の線量評価を考えるうえで重要なのは計算される線量の考え方である．内部被曝は預託実効線量で示す．放射性物質の摂取後に，体内に残留している放射性物質から個々の組織または臓器が受ける線量率を時間積分した線量である．図 1 に預託実効線量の考え方を示す．成人ならば摂取後 50 年間，小児の場合は 70 歳までに被曝する積算量をいい，この線量を 1 年間ですべて被曝すると仮定して計算するが，実際はまだ被曝していない線量も含むことになる．預託等価線量(臓器ごとの内部被曝線量)の単位もシーベルト Sv である．

内部被曝放射線の計測法
——WBCを中心として

体内被曝が生じた場合，治療の必要性の有無と予後を判定するためには摂取された核種を同定し，被曝量を推定する．そのためにはまず体内に存在している放射性物質の放射能(ベクレル，Bq)を求める必要がある．

体内の放射能の推定方法には大きく分けて次の 3 種類の方法がある．①鼻腔スメアー法，②バイオアッセイ法，③体外計測法である．内部被曝の可能性を知るために，鼻腔に濾紙や綿棒を挿入し，粘膜に付着した放射性物質を同定し，またその量から吸入量を推定する方法がある．これが鼻腔スメアー法であり，もっとも簡易な方法であるが，誤差も大きいため，内部被曝の有無のためのスクリーニングとして使われることが多い．バイオアッセイ法はおもに排泄物中の放射能量を経時的に定量し，体内に摂取された放射能量を推定す

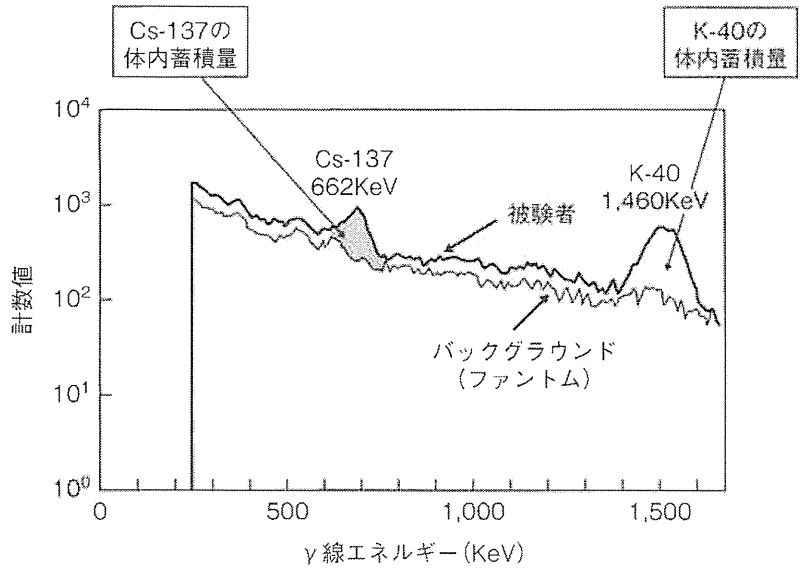


図 3　WBCのスペクトラム

横軸はγ線のエネルギー(KeV，キロエレクトロンボルト)，縦軸は計数値(頻度)を示す．被検者に Cs-137 による 662Kev のピークが観察された例を示す．

る方法である．1日に排泄される放射能から，あるモデルを使って体内に摂取された量を推定する．αやβ核種など透過性の小さな放射性物質による体内被曝の場合，この方法により評価を行うことが多い．

1．体外計測法

体外計測法は体内に摂取された放射性物質から放出される透過性放射線を体外で検出し，そのエネルギーから放射性物質の同定と量を測定する方法である．したがって，γ線あるいはX線を放出する核種が対象となり，αあるいはβ線のみを放出する核種を検出することはできない．ホールボディカウンタ(whole body counter：WBC)は全身に分布する放射性物質を測定する装置で，放射線の検出はヨウ化ナトリウム(NaI(Tl))などのシンチレータやゲルマニウム(Ge)半導体を用いた検出器により放射性物質からのエネルギーを分析し，放射性物質の同定を行う．後者はエネルギーの分解能が優れており，核種の同定に高い精度をもつ(図2)．

2．WBCの原理

図3にWBCの実際の測定スペクトルを示す．横軸(チャネル)をさまざまなエネルギーの放射線を用いて対応させることをエネルギー校正という．これを行うことで横軸をエネルギーで表示することができる．また，頻度を放射線量と対応させることを効率校正という．これを行うことにより頻度から放射線の量，したがって，それを放出している放射性物質の量を知ることができる．エネルギー校正が正しく行われていないと，また放出されるγ線のエネルギー情報が正しくないと，スペクトルから放射性物質の種類を同定することは困難となる．エネルギー校正は放出γ線のエネルギーがわかっている放射性同位元素が数種類あれば実行できる．効率校正は測定する試料の形状にあっており，既知量の放射性物質をもった線源が必要である．ヒトなどを測る場合は，ヒト型をした大きな線源が必要である．図4にヒト用校正線源の例を示す．この校正が行われていない

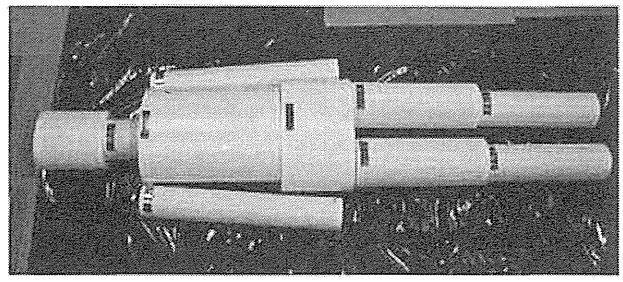


図 4　体外計測用のヒトを模した体積線源(BOMABファントム)

測定する試料の形状をしており，既知量の放射性物質が入っている．効率校正には不可欠である．

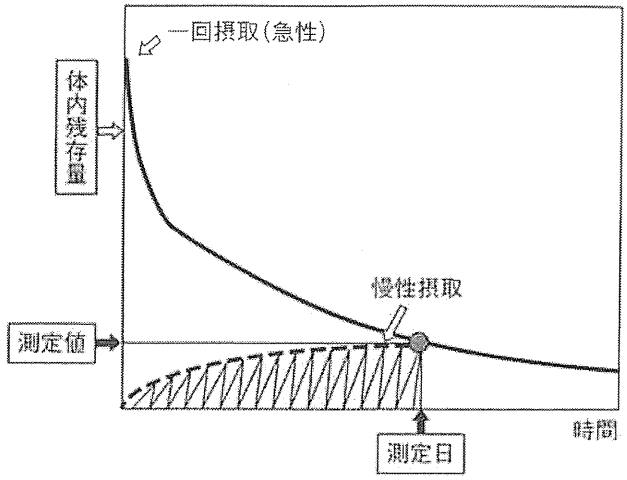


図 5　内部被曝の線量評価
一度に摂取した場合と慢性的に摂取された際の体内残存量を模式的に示す．時間に沿って積分した数値をもとに，内部被曝線量を計算する．

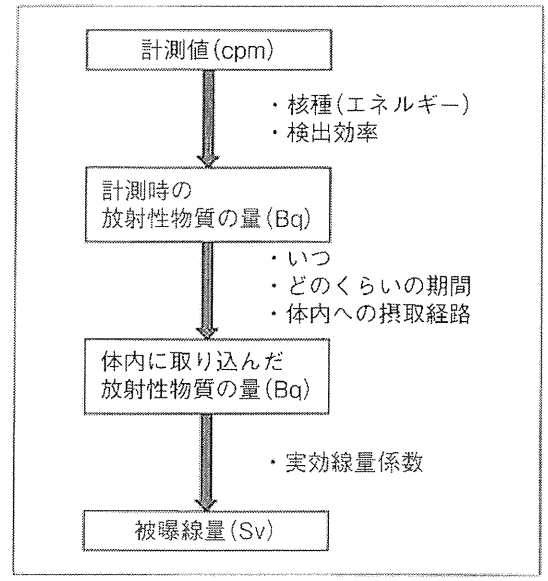


図 6　体外計測から内部被曝線量評価まで
体外計測により得られた計数(cpm)は，エネルギーと機器の検出効率などから，測定時に体内に残存している放射能に換算される(Bq)．さらに，摂取経路，時期などから摂取量が推定される(Bq)．線量係数に基づき，被曝線量が評価される(Sv)．

WBC は放射性物質の同定を誤らせ，過大あるいは過小な線量を導き出す．WBC 測定には技術と経験を要する．

WBC が設置されている周囲の構造物や壁からはつねに自然放射線が放出されている．これをバックグラウンド放射線という(図 3)．被検者の測定で得られた放射線の計測値は体内からの放射線とバックグラウンド放射線との和として測定される．微量の放射線は小さなピークとして検出されるため，微量の放射性物質や放出頻度の低い放射線を測定するためには，遮蔽によるバックグラウンド放射線の低減化は重要である．

WBCによる計測の実際

WBC は体表面と体内の放射性物質を区別できないため，被検者の衣服や体表面に放射性物質の汚染があると正確な計測はできない．事前にシャワーを浴びるか着替えるかなどの除染が必要となる．WBC により得られた放射線カウント(count per minute：cpm)は放射性物質のエネルギーと機器の検出効率などから，体内の放射能(Bq)に換算される．つまり WBC でわかるのは測定時に体内に残存している放射性物質の種類と放射能までである．つぎに行うのが線量推定であるが，線量について WBC は教えてくれない．

内部被曝の線量評価

WBC などから摂取された放射性物質の量(Bq)がわかることは上述のとおりである．しかし，放射性物質が体内に取り込まれた場合，経口摂取なのかあるいは吸入なのか，その経路，一度に摂取されたのか(急性摂取)，徐々になのか(慢性摂取)，化学形などによって，循環血液中や臓器への移行する割合が異なる．繰り返すが，WBC でわかるのは測定した時点で体内に存在する放射能(Bq)である．内部被曝線量は摂取された放射性物質の量をもとに計算するため，摂取した日，摂取経路から計算することになる．経口であれば，消化管からの吸収率，吸入であれば粒子径などの性質を放射性物質ごとに考えることが要求される．

これらの因子と実効半減期から線量へと換算する係数が，国際放射線防護委員会(International Committee of Radiation Protection：ICRP)の“Publication 68”(作業者)[4]，72(一般公衆)[5]に掲載されている．この係数を用いて測定日までの日数，摂取経路などの摂取シナリオを決定して，はじめて被曝線量(Sv)の計算ができる．この計算シナリオが異なれば，体外計測で同じ残存量であっても線量は大きく異なる．図 5 に一度に摂取した

表 3 “ICRP Publication 89”[7]による1 mSvの内部被曝に相当する尿中セシウム(Cs)量

核種		成人・男 (18歳以上) 尿量 1.6 L/day	成人・女 (18歳以上) 1.2 L/day	15歳 (13歳以上 18歳未満) 1.2 L/day	10歳 (8歳以上 13歳未満) 0.7 L/day	5歳 (3歳以上 8歳未満) 0.5 L/day
Cs-134	(Bq/day)	91	91	91	78	31
	(Bq/L)	57	76	76	111	62
	尿量が3倍* (Bq/L)	19	25	25	37	21
Cs-137	(Bq/day)	146	146	145	125	51
	(Bq/L)	91	122	121	179	102
	尿量が3倍* (Bq/L)	30	41	41	60	34

平成23年3月12日に急性1回吸入，同年7月20日に尿中測定を行ったと仮定し，計算ソフトウェアMONDAL[8]を使って計算した．
*：“ICRP Publication 89”が示す尿量の3倍の尿量とした場合の尿中のCs濃度を示した．

場合と毎日慢性的に摂取された際の体内残存量を模式的に示す．縦軸は放射性物質の残存量で，横軸は時間である．内部被曝線量は「体内を通り過ぎて行った放射性物質からの放射線」に反映されるので，積分した数値が被曝線量となる．線量は摂取方法により大きく異なることがわかる．図6に，WBCによる体外計測から体内被曝線量までの過程を示した．

東京電力福島第一原子力発電所の事故

今回の東京電力福島第一原子力発電所の事故では放射線医学総合研究所(放医研)と日本原子力研究開発機構(JAEA)が一部住民の内部被曝線量評価のため，WBCによる測定を実施した．放医研で測定した平成23年(2011)7月10日までの結果が福島県のホームページで公開されている[6]．放医研では，放射性物質の環境への放出がはじまった平成23年3月12日に急性1回吸入したという線量が高く計算されるシナリオで線量を計算した．しかし，結果として109名のうち1名も1 mSvを超えなかったのはホームページの掲載のとおりである．

同時に1回の随時尿中のセシウム(Cs)-134，Cs-137を測定した．“ICRP Publication 89”は，1日に尿中に排泄されるCsの量から，内部被曝線量評価法を示している[7]．今回の尿検査は随時尿であり，24時間畜尿と比べ線量評価の誤差は大きく，線量評価には適当ではないが，平成23年3月12日の急性1回吸入，同7月10日に尿中の測定をしたと仮定し，内部被曝線量が1 mSvとなるために必要な，24時間尿で排泄されるCs-134，Cs-137の量を計算してみた(表3)[8]．成人ではCs-137とCs-134の1日の尿排泄量が91 Bqと146 Bqにもなる．この方法では年齢ごとの24時間尿量が示されているため，1 L(リットル)中のCs濃度が計算できる．公開された成人の随時尿中のCs濃度に比べ，“ICRP Publication 89”による尿中Cs平均濃度は非常に高いことがわかる．随時尿中のCs濃度は水分摂取量や発汗などにより影響するため，“ICRP Publication 89”に示された3倍の尿量とした場合を想定した濃度と比べても，測定値のほうが明らかに低い．

おわりに

内部被曝の線量評価は測定時に体内に存在する放射能から，ある種の仮定のもとに計算する．正確な摂取時期や経路が特定できないかぎり曖昧な点が残るため，過大評価になることが多い．医療の観点からは，行うべき治療を行わないことを避けることを考慮すれば，過小評価になってはならない．しかし，事故から7カ月以上たち，放射性物質を吸入する可能性が非常に低い状態が続く現在，平成23年3月12日の急性1回吸入とするシナリオによる“安全側”“保守的”な線量評価は，科学的に矛盾点が多く，かえって住民に誤った情報を与えることになり，不安感が増すばかりであ

る．WBCによって得られた内部被曝線量を正しく理解し，正しいWBCの利用が望まれる．

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