



(86) Date de dépôt PCT/PCT Filing Date: 2000/06/22
(87) Date publication PCT/PCT Publication Date: 2000/12/28
(45) Date de délivrance/Issue Date: 2012/09/25
(85) Entrée phase nationale/National Entry: 2001/12/19
(86) N° demande PCT/PCT Application No.: JP 2000/004095
(87) N° publication PCT/PCT Publication No.: 2000/079280
(30) Priorité/Priority: 1999/06/22 (JP11/175928)

(51) Cl.Int./Int.Cl. *C12N 15/40* (2006.01),
C07K 14/08 (2006.01), *C07K 16/10* (2006.01),
G01N 33/543 (2006.01), *G01N 33/569* (2006.01)

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(54) Title: SRSV DETECTION KIT

(57) **Abrégé/Abstract:**

This invention relates to an SRSV detection kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide groups (a) to (k), respectively: (a) a peptide having an amino acid sequence represented by SEQ ID NO: 1, and the like, (b) a peptide having an amino acid sequence represented by SEQ ID NO : 2, and the like, (c) a peptide having an amino acid sequence represented by SEQ ID NO : 3, and the like, (d) a peptide having an amino acid sequence represented by SEQ ID NO : 4, and the like, (e) a peptide having an amino acid sequence represented by SEQ ID NO : 5, and the like, (f) a peptide having an amino acid sequence represented by SEQ ID NO : 6, and the like, (g) a peptide having an amino acid sequence represented by SEQ ID NO: 7, and the like, (h) a peptide having an amino acid sequence represented by SEQ ID NO: 8, and the like, (i) a peptide having an amino acid sequence represented by SEQ ID NO : 9, and the like, (j) a peptide having an amino acid sequence represented by SEQ ID NO: 10, and the like, and (k) a peptide having an amino acid sequence represented by SEQ ID NO: 11, and the like. Use of the kit makes it possible to detect most SRSV-related viruses and further to distinguish their serotypes and genogroups, easily and surely.

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ABSTRACT

This invention relates to an SRSV detection kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide groups (a) to (k),
5 respectively: (a) a peptide having an amino acid sequence represented by SEQ ID NO: 1, and the like, (b) a peptide having an amino acid sequence represented by SEQ ID NO: 2, and the like, (c) a peptide having an amino acid sequence represented by SEQ ID NO: 3, and the like, (d) a peptide having an amino acid sequence
10 represented by SEQ ID NO: 4, and the like, (e) a peptide having an amino acid sequence represented by SEQ ID NO: 5, and the like, (f) a peptide having an amino acid sequence represented by SEQ ID NO: 6, and the like, (g) a peptide having an amino acid sequence represented by SEQ ID NO: 7, and the like, (h) a peptide having
15 an amino acid sequence represented by SEQ ID NO: 8, and the like, (i) a peptide having an amino acid sequence represented by SEQ ID NO: 9, and the like, (j) a peptide having an amino acid sequence represented by SEQ ID NO: 10, and the like, and (k) a peptide having an amino acid sequence represented by SEQ ID NO: 11, and
20 the like.

Use of the kit makes it possible to detect most SRSV-related viruses and further to distinguish their serotypes and genogroups, easily and surely.

DESCRIPTION**SRSV DETECTION KIT****5 Technical Field**

 This invention relates to a kit for detecting and distinguishing one or more small round structure viruses (hereinafter called "SRSVs") in a specimen.

Background Art

10 SRSVs are a group of causative viruses of human viral gastroenteritis, the discovery of the first one of which goes back to 1972. They are known to cause infantile acute gastroenteritis and also outbreaks of food poisoning or the like among adults and preschool or elementary school children. Due
15 to the inability to proliferate these SRSVs by cell culture and the lack of animal models capable of exhibiting sensitivity thereto, SRSV antigens and anti-SRSV antibodies are hardly available, resulting in a delay in the development of immunoserologic methods for the detection of the viruses.

20 Under such circumstances, it was succeeded to clone the gene of the Norwalk virus, an SRSV, in 1993, leading to the determination of the base sequence of its complete genomes [JP(PCT) 6-506823 A]. Subsequently, PCR methods which are useful to amplify a part of an RNA polymerase region were developed,
25 and 14 SRSV-related viruses have been found to date. As a result

of analyses of about 120 amino acids in these RNA polymerase regions, SRSVs are considered to be roughly differentiated into two genogroups, that is, Genogroup I including the Norwalk virus strain as a prototype and Genogroup II including the Snow Mountain virus strain as a prototype.

As genetic analyses of SRSV-related viruses proceeded, it came to knowledge that substantial diversity exists even in the same genogroup. As a matter of fact, it was found that with an RT-PCR method making use of primers for the genes of the Norwalk virus and Snow Mountain virus strains as the prototypes of the respective genogroups, every SRSV is not detectable and also that it is very difficult to design primers or set RT-PCR conditions for achieving efficient amplification of SRSVs.

In the meantime, antigens were prepared against some of the viruses, such as the Norwalk virus strain and the Snow Mountain strain, by genetic expression, antibodies were obtained, and ELISA-dependent SRSV detection methods making use of such antibodies were also developed. It was, however, still impossible to detect every gastroenteritis-causing SRSV due to the diversity of the SRSVs.

In Japan, on the other hand, SRSVs were designated in 1997 to be causative factors of food poisoning as defined in the Food Sanitation Act so that, if SRSV food poisoning breaks out, determination of its infection route is required. There is accordingly a desire for a method which easily and surely detects

and identifies SRSVs in infected subjects' feces or foods.

Disclosure of the Invention

Accordingly, an object of the present invention is to
5 provide a kit which can easily detect from a specimen an
SRSV-related virus known to date and can surely discriminate
its serotype and genogroup.

With the foregoing circumstances in view, the present
inventors have proceeded with an genetic and immunological
10 investigation on SRSV-related viruses. As a result, it has been
found that combined use of antibodies obtained from 11
SRSV-related virus peptides, including newly-found novel virus
peptides, can detect most SRSVs in specimens and can surely
discriminate the serotypes and genogroups of the SRSVs, leading
15 to the completion of the present invention.

Specifically, the present invention provides an SRSV
detection kit comprising all antibodies against SRSV-related
virus constituting peptides selected from the following peptide
groups (a) to (k), respectively:

20 (a) a peptide having an amino acid sequence represented
by SEQ ID NO: 1 and peptides each having at least 80% of homology
with said amino acid sequence, and partial peptides thereof,

(b) a peptide having an amino acid sequence represented
by SEQ ID NO: 2 and peptides each having at least 80% of homology
25 with said amino acid sequence, and partial peptides thereof,

(c) a peptide having an amino acid sequence represented by SEQ ID NO: 3 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

5 (d) a peptide having an amino acid sequence represented by SEQ ID NO: 4 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(e) a peptide having an amino acid sequence represented by SEQ ID NO: 5 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

10 (f) a peptide having an amino acid sequence represented by SEQ ID NO: 6 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(g) a peptide having an amino acid sequence represented by SEQ ID NO: 7 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

15 (h) a peptide having an amino acid sequence represented by SEQ ID NO: 8 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(i) a peptide having an amino acid sequence represented by SEQ ID NO: 9 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

20 (j) a peptide having an amino acid sequence represented by SEQ ID NO: 10 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

25 and

(k) a peptide having an amino acid sequence represented by SEQ ID NO: 11 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof.

The present invention also provides an SRSV detection kit
5 for discriminating SRSVs in genogroup, said SRSV detection kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide groups (a) to (d), respectively:

(a) a peptide having an amino acid sequence represented
10 by SEQ ID NO: 1 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(b) a peptide having an amino acid sequence represented by SEQ ID NO: 2 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

15 (c) a peptide having an amino acid sequence represented by SEQ ID NO: 3 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof, and

(d) a peptide having an amino acid sequence represented
20 by SEQ ID NO: 4 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof.

Further, the present invention also provides an SRSV detection kit for discriminating genogroup of SRSVs, said SRSV detection kit comprising all antibodies against SRSV-related
25 virus constituting peptides selected from the following peptide

groups (e) to (k), respectively:

(e) a peptide having an amino acid sequence represented by SEQ ID NO: 5 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

5 (f) a peptide having an amino acid sequence represented by SEQ ID NO: 6 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(g) a peptide having an amino acid sequence represented by SEQ ID NO: 7 and peptides each having at least 80% of homology
10 with said amino acid sequence, and partial peptides thereof,

(h) a peptide having an amino acid sequence represented by SEQ ID NO: 8 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(i) a peptide having an amino acid sequence represented
15 by SEQ ID NO: 9 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(j) a peptide having an amino acid sequence represented by SEQ ID NO: 10 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,
20 and

(k) a peptide having an amino acid sequence represented by SEQ ID NO: 11 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof.

Furthermore, the present invention also provides
25 SRSV-related virus strain genes having base sequences

represented by SEQ ID NOS: 15, 20, 21 and 22 or base sequences similar to the first-mentioned base sequences, respectively, except for deletion, replacement or addition of one to several bases of said first-mentioned base sequences.

5

Brief Description of the Drawings

FIG. 1 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Seto 124/1989/JP strain.

10 FIG. 2 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Funabashi 258/1996/JP strain.

FIG. 3 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Chiba 407/1987/JP strain.

FIG. 4 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Narita 104/1997/JP strain.

15 FIG. 5 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Sanbu 809/1998/JP strain.

FIG. 6 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Ichikawa 754/1998/JP strain.

20 FIG. 7 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Chitta 1876/1996/JP strain.

FIG. 8 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Kashiwa 47/1997/JP strain.

FIG. 9 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Mie 7k/1994/JP strain.

25 FIG. 10 is an electron micrograph (x 100,00) of virus-like

particles derived from the Hu/NLV/Kashiwa 645/1999/JP strain.

FIG. 11 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Osaka 10-25/1999/JP strain.

5 **Best Modes for Carrying Out the Invention**

1. SRSV-related viruses

The SRSV detection kit according to the present invention is characterized by the use of the antibodies against SRSV-related virus constituting peptides having the 11 specific amino acid sequences or at least 80% of homologies with the amino acid sequences in the groups (a) to (k). Of these, the peptides belonging to the group (d), the group (i), the group (j) and the group (k) are novel peptides different from any SRSV-related viruses registered with the GeneBank to date (Table 1, which will be described subsequently herein). Owing to the incorporation of the 11 antibodies, including antibodies against these novel peptides, into the kit, SRSV-related viruses can be detected without omission.

The SRSV-related virus constituting peptides useful in the present invention embrace their mutants in each of which one or more amino acids have been deleted from, replaced in or added to its corresponding amino acid sequence; and also their mutants in each of which one or several bases have been deleted from, replaced in or added to a base sequence encoding its corresponding amino acid sequence.

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 1 in the group (a) is a virus constituting peptide of the Hu/NLV/Kashiwa 645/1999/JP strain obtained from feces of an SRSV infected patient in Japan, whereas
5 examples of the peptides each having at least 80% of homology with the amino acid sequence include one derived from the Desert Shield/90/SA strain (GeneBank Accession No. U04469).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 2 in the group (b) is a virus
10 constituting peptide of the Hu/NLV/Seto 124/1989/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include those derived from the KY-89/89J strain (GeneBank Accession No. L23828) and the
15 Norwalk/68/US strain (GeneBank Accession No. M876611).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 3 in the group (c) is a virus constituting peptide of the Hu/NLV/Funabashi 258/1996/JP strain obtained from feces of an SRSV infected patient in Japan, whereas
20 examples of the peptides each having at least 80% of homology with the amino acid sequence include one derived from the Southampton/91/UK strain (GeneBank Accession No. L07418).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 4 in the group (d) is a virus
25 constituting peptide of the Hu/NLV/Chiba 407/1987/JP strain

obtained from feces of an SRSV infected patient in Japan.

The peptide having the amino acid sequence represented by SEQ ID NO: 4 has less than 75% of homology in structural gene (SEQ ID NO: 15) with any one of the SRSV-related virus strains (Table 1, which will be described subsequently herein) registered with the GeneBank to date, and is a peptide having a novel sequence not reported to date.

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 5 in the group (e) is a virus constituting peptide of the Hu/NLV/Narita 104/1997/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include those derived from the Bristol/93/UK strain (GeneBank Accession No. X76716), the Lordsdale/93/UK strain (GeneBank Accession No. X86557), and the Camberwell/94/AU strain (GeneBank Accession No. U46500).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 6 in the group (f) is a virus constituting peptide of the Hu/NLV/Sanbu 809/1998/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include those derived from the Mexico/89/MEX strain (GeneBank Accession No. U22498), the Auckland strain (GeneBank Accession No. U460391), the Toronto/77/CA strain (GeneBank Accession No. U02030), and the

OTH-25/89/J strain (GeneBank Accession No. L23830).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 7 in the group (g) is a virus constituting peptide of the Hu/NLV/Ichikawa 754/1998/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include those derived from the Snow Mountain/76/US strain (GeneBank Accession No. U70059) and the Melksham/89/UK strain (GeneBank Accession No. X81879).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 8 in the group (h) is a virus constituting peptide of the Hu/NLV/Chitta 1876/1996/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include one derived from the Hawaii/71/US strain (GeneBank Accession No. U07611).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 9 in the group (i) is a virus constituting peptide of the Hu/NLV/Kashiwa 47/1997/JP strain obtained from feces of an SRSV infected patient in Japan.

The peptide having the amino acid sequence represented by SEQ ID NO: 9 has less than 75% of homology in structural gene (SEQ ID NO: 20) with any one of the SRSV-related virus strains (Table 1, which will be described subsequently herein) registered with the GeneBank to date, and is a peptide having a novel sequence

not reported to date.

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 10 in the group (j) is a virus constituting peptide of the Hu/NLV/Mie 7k/1994/JP strain
5 obtained from feces of an SRSV infected patient in Japan.

The peptide having the amino acid sequence represented by SEQ ID NO: 10 has less than 70% of homology in structural gene (SEQ ID NO: 21) with any one of the SRSV-related virus strains (Table 1, which will be described subsequently herein) registered
10 with the GeneBank to date, and is a peptide having a novel sequence not reported to date.

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 11 in the group (k) is a virus constituting peptide of the Hu/NLV/Osaka 10-25/1999/JP strain
15 obtained from feces of an SRSV infected patient in Japan.

The peptide having the amino acid sequence represented by SEQ ID NO: 11 has less than 70% of homology in structural gene (SEQ ID NO: 22) with any one of the SRSV-related virus strains (Table 1, which will be described subsequently herein) registered
20 with the GeneBank to date, and is a peptide having a novel sequence not reported to date.

Table 1

Virus strain	GeneBank Accession No.
Desert Shield/90/SA	U04469
Norwalk/68/US	M876611
KY-89/89J	L23828
OTH-25/89/J	L23830
Southampton/91/UK	L07418
Lordsdale/93/UK	X86557
Bristol/93/UK	X76716
Camberwell/94/AU	U46500
Toronto/77/CA	U02030
Mexico/89/MEX	U22498
Snow Mountain/76/US	U70059
Melksham/89/UK	X81879
Auckland	U460391
Hawaii/71/US	U07611

The SRSV-related virus constituting peptides in these groups (a) to (k) embrace, in addition to the above-described peptides, partial peptides each of which contains a specific amino acid sequence in its corresponding peptide and has antigenecity equivalent to the corresponding peptide.

According to a homological analysis of about 120 amino acids of RNA polymerase regions of the SRSV-related virus constituting peptides, these SRSV-related virus constituting peptides can be classified into two genogroups. Described

specifically, they can be classified into Type I to which the peptides in the groups (a) to (d) belong and Type II to which the peptides in the groups (e) to (k) belong.

2. Cloning of the SRSV-related virus constituting genes

5 From feces of an SRSV infected patient, viral RNA is extracted using the cetyltrimethylammonium bromide (CTAB) method or the like, cDNA was formed by an oligo-dT primer and a reverse transcriptase, and using the cDNA and primers capable of amplifying structural gene regions of the individual
10 SRSV-associate viruses, PCR was conducted to amplify structural gene fragments.

Such a structural gene fragment is inserted in a plasmid by once conducting TA cloning with an *E. coli* cloning vector.

As a cloning vector usable here, it is possible to use
15 a known cloning vector such as a vector derived from a plasmid obtained using as host procaryotic cells represented by *E. coli* or from a bacteriophage represented by ϕ phage, and appropriately combined use of a cloning vector and its host cell is desired. Specific examples of the cloning vector include pBR322, pUC19
20 and pCRII. The insertion of the DNA can be conducted by a method known *per se* in the art, and upon formation of such a vector, use of *E. coli* cells is desired as they permit easy genetic manipulation.

3. Expression of structural gene and creation of virus-like 25 particles.

By having fragments of the above-obtained individual virus constituting genes in the groups (a) to (k) expressed with a suitable expression system or by using virus-like particles created from the virus constituting peptides in a genetic engineering manner, antibodies against the respective viruses can be obtained. A description will hereinafter be made about an expression when *E. coli* is used and also about the creation of virus-like particles.

(1) Expression by *E. coli*

Plasmids with the structural gene regions of the respective SRSV-related viruses incorporated therein, respectively, are each digested with a restriction endonuclease which does not cleave the structural gene region. Then, the structural gene region is collected and incorporated, for example, in pGEX (GST fusion protein expression vector; product of Pharmacia AB), pTrc99A (*E. coli* expression vector; product of Pharmacia AB), pTrxFus (thioredoxin fusion protein expression vector; product of Invitrogen Corporation), pET (expression vector making use of pT7RNA promoter; product of Novagen Inc.), a maltose binding protein expression vector, or a α galactosidase fusion protein expression vector. At this time, the structural gene region to be incorporated can be of the complete length or can be a partial region, with a partial region containing at least one antigen epitope of an SRSV being preferred. Gene expression vectors with the structural gene regions incorporated therein

as described above are transformed by an *E coli* strain suited for gene expression, for example, the BL21 strain, the DH10B strain, the JM109 strain or the XL1-Blue strain. Expression of the gene can be conducted by culturing the thus-obtained transformants in a general liquid culture medium, for example, L-broth. It is preferred for the expression to add a gene expression promoter, for example, IPTG or, when a PL promoter is used, to apply a heat shock.

Purification of a peptide so expressed can be conducted following a general purification method for expressed protein, which makes use of *E coli*. If the expressed protein is in a dissolved form, for example, its purification can be conducted by affinity chromatography making use of a GST column or a column for maltose binding proteins. If the expressed protein is in an insoluble form, its purification can be achieved by conducting affinity chromatography making use of a Ni chelate.

(2) Creation of SRSV virus-like particles

A plasmid with a structural gene region of an SRSV-related virus incorporated therein is digested with a restriction endonuclease which does not cleave the structural gene region. Then, the structural gene region is collected and incorporated, for example, in a baculovirus transfer vector such as pVL1393. The transfer vector and a linear baculovirus DNA, from which a gene region essential for proliferation has been deleted, are subjected to transfection in insect cells such that homologous

recombination is induced to form the target recombinant baculovirus.

By infecting the thus-obtained recombinant baculovirus to insect cells such as Sf9 cells or Tn5 cells and incubating the infected insect cells under adequate growth conditions in a manner known *per se* in the art, the structural protein of the SRSV is expressed. By allowing the structural protein to undergo self-assembly, virus-like particles can be produced. Use of a biochemical purification method, for example, centrifugation makes it possible to isolate and purify the virus-like particles. Whether or not such virus-like particles have been formed can be confirmed by subjecting the self-assembled product to negative staining with uranyl acetate and examining the stained self-assembled product by an electron microscope.

The virus-like particles obtained as described above do not have infectiveness as they do not contain any gene internally. Nonetheless, they have antigenicity equivalent to virus particles because they structurally have substantially the same shape as virus particles.

4. Acquisition of antibodies against SRSV-related viruses

By immunizing an animal with the thus-obtained virus constituting peptide or virus-like particles, an anti-SRSV-related virus antibody can be prepared. Incidentally, such an antibody can be either a monoclonal antibody or a polyclonal antibody.

Preparation of an immune antibody by making use of virus-like particles can be conducted, for example, as will be described next. In a manner known *per se* in the art, a rabbit is immunized with virus-like particles of one of the SRSV-related viruses, and from separated serum, an IgG antibody (anti-SRSV antibody) against the virus-like particles can be obtained. For the separation and isolation of the antibody, a method such as DEAE Sepharose chromatography can be used.

Using the 11 types of virus-like particles of the groups (a) to (k) obtained as described above and their corresponding anti-SRSV antibodies, their cross reactivities were measured. As will be shown below in Table 2, absolutely no cross-reactivity was exhibited between the individual SRSV-related viruses. According to the SRSV detection method of the present invention, it is therefore possible to concurrently discriminate the serotypes of 11 types of SRSVs. This also indicates the possibility of discriminating Genogroup I Genogroup II from each other at the same time.

5. Detection of SRSV-related viruses

For the detection of one or more SRSVs in a specimen by the individual anti-SRSV antibodies obtained as described, conventionally employed immunoassays making use of antigen-antibody reactions, for example, radioimmunoassay by the sandwich technique, enzyme-linked immunosorbent assay (ELISA) and the like can be used, with ELISA being particularly

preferred. Described specifically, the 11 types of anti-SRSV antibodies are separately poured into a microplate to prepare an SRSV screening plate. A dilution of a fecal emulsion, which has been prepared from feces of an SRSV infected patient, is added to the wells of the plate, and is then allowed to react. Peroxidase (POD) labeled anti-SRSV antibodies of the respective viruses are thereafter added and reacted. After a substrate solution (TMB containing hydrogen peroxide) is added and reacted, 0.6 N sulfuric acid is added to stop the reactions. By measuring the absorbance (450 nm/630 nm) of each well by an ELISA autoreader, the SRSV or SRSVs can be detected.

When it is desired to conduct only the detection of one or more SRSVs in a specimen, a detection kit can be prepared by using a microplate with all the 11 types of anti-SRSV antibodies mixed and immobilized thereon. To also discriminate even the serotypes of the one or more SRSVs, a detection kit can be prepared by using microplates with all the 11 types of anti-SRSV antibodies immobilized separately thereon.

Further, the discrimination of the genogroups is feasible by a kit making use of a microplate with antibodies against the peptides in the groups (a) to (d) mixed and immobilized thereon (Type I plate) or a microplate with antibodies against the peptides in the groups (e) to (k) mixed and immobilized thereon (Type II plate).

Moreover, immobilization of the individual anti-SRSV

antibodies useful in the present invention with a carrier such as a latex or magnetic beads makes it possible to surely capture one or more SRSV-related viruses in a specimen. The carrier with one or more SRSV-associate viruses captured thereon can be recovered by centrifugation in the case of the latex or by magnet in the case of the magnetic beads. Subsequent to the recovery, virus RNAs can be extracted and used.

Examples

The SRSV detection kits according to the present invention will hereinafter be specifically described based on Examples. Example 1 Cloning of structural genes of SRSV-related viruses

(1) Synthesis of cDNA

PBS (9 mL) and "Daiflon" (1 mL) were added to feces (0.5 to 1.0 g) of an SRSV patient, followed by homogenization. The homogenate was then centrifuged at 3,000 rpm for 20 minutes, and the supernatant was collected as a 10% fecal emulsion.

Using a 1-mL aliquot of the fecal emulsion, RNA of the SRSV was extracted by the cetyltrimethylammonium bromide (CTAB) method, and the RNA was eventually suspended in a 0.1% diethyl pyrocarbonate solution (30 μ L). Using the suspension, cDNA was prepared by a reverse transcriptase derived from the Oligo-dT(12-18) primer and AMV (Avian Myeloblastosis Virus) (product of SEIKAGAKU CORPORATION).

(2) Isolation of structural gene regions

Using the cDNA prepared in (1) and primers for amplifying the structural gene regions shown below, PCR was conducted. Subsequent to the PCR, amplified structural gene fragments were separated by agarose gel electrophoresis, and were then recovered by using "SuprecTM-01" (TAKARA).

Hu/NLV/Kashiwa 645/1999/JP gene:G1/F2(SEQ ID NO: 23),
Oligo-dT(33) (SEQ ID NO: 24)

Hu/NLV/Seto 124/1989/JP gene:G1/F2(SEQ ID NO: 23),G1/R0
(SEQ ID NO: 25)

Hu/NLV/Funabashi 258/1996/JP gene:G1/F2(SEQ ID NO: 23),
Oligo-dT(33)(SEQ ID NO: 24)

Hu/NLV/Chiba 407/1987/JP gene:D5(SEQ ID NO: 26),
CV-U4(SEQ ID NO: 27)

Hu/NLV/Narita 104/1997/JP gene:97k104/F1(SEQ ID NO: 28),
97k104/R1(SEQ ID NO: 29)

Hu/NLV/Sanbu 809/1998/JP gene:G2/F3(SEQ ID NO: 30),
MV-R1(SEQ ID NO: 31)

Hu/NLV/Ichikawa 754/1998/JP gene:G2/F3(SEQ ID NO: 30),
SMV-R1(SEQ ID NO: 32)

Hu/NLV/Chitta 1876/1996/JP gene:G2/F3(SEQ ID NO: 30),
G2/R0(SEQ ID NO: 33)

Hu/NLV/Kashiwa 47/1997/JP gene:97k104/F1(SEQ ID NO: 28),
Oligo-dT(33)(SEQ ID NO: 24)

Hu/NLV/Mie 7k/1994/JP gene:G2/F3(SEQ ID NO: 30),
Oligo-dT(33)(SEQ ID NO: 24)

Hu/NLV/Osaka 10-25/1999/JP gene:GFCR7(SEQ ID NO: 34),
Oligo-dT(33)(SEQ ID NO: 24)

(3) Cloning of structural genes

TA cloning of the recovered structural gene fragments to
5 an *E. coli* cloning vector, pCRII(product of Invitrogen
Corporation) was conducted. Obtained from these clones were
plasmids with the structural genes of the viruses incorporated
therein, pCRII/645, pCRII/124, pCRII/258, pCRII/Chiba,
pCRII/104, pCRII/809, pCRII/754, pCRII/76, pCRII/47, pCRII/7k,
10 and pCRII/10-25.

Example 2 Determination of base sequences

Determination of the base sequences of the structural genes
of the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto
124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain,
15 the Hu/NLV/Chiba 407/1987/JP strain, the Hu/NLV/Narita
104/1997/JP strain, Hu/NLV/Sanbu 809/1998/JP strain, the
Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta
1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the
Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka
20 10-25/1999/JP strain was conducted in the below-described
manner.

Firstly, a primer (first primer) was set in the vicinity
of the polyhedrin promoter of pVL1393 as a transfer vector, and
by the dye termination method, a labeling reaction was conducted
25 by using a "Cycle Sequencing Kit FS" (product of Perkin-Elmer

Corp.). The DNA concentration of the transfer vector employed was 0.4 μ g/ μ L, whereas the concentration of the sequencing primer used was 3.2 pmol/ μ L. Subsequent to the reaction, the excess fluorescent pigment was eliminated using a centriprep spin column (manufactured by Perkin-Elmer Corp.). The reaction mixture was completely dried by a vacuum lyophilizer, and the lyophilizate was suspended in a special sample buffer (20 μ L; product of Perkin-Elmer Corp.). Subsequent to stirring, the suspension was subjected to centrifugal precipitation. The precipitate was dried at 95°C for 2 minutes. After quenching, it was analyzed by an autosequencer ("ABI Genetic Analyzer 310").

Using the base sequence determined by the first primer, a new sequencing primer (second primer) was set on the 3'-side of the base sequence. Using this second primer, a labeling reaction was conducted by a cyclic sequencing kit in a similar manner as mentioned above. Subsequent to the reaction, operation similar to that mentioned above was performed, and the base sequence was analyzed by the autosequencer. As has been described above, a sequencing primer was set on the 3' side of the base sequence determined in each cycle, and determination of the base sequence was conducted. By repeating this procedure, the base sequences from the 5'-ends to the 3'-ends of the 11 types of SRSV-related virus structural genes (SEQ ID NO: 12 to SEQ ID NO 22) were determined. Among these, the base sequences represented by SEQ ID NO: 15 (the Hu/NLV/Chiba 407/1987/JP

strain), SEQ ID NO: 20 (the Hu/NLV/Kashiwa 47/1997/JP strain),
SEQ ID NO: 21 (the Hu/NLV/Mie 7k/1994/JP strain) and SEQ ID
NO: 22 (the Hu/NLV/Osaka 10-25/1999/JP strain) were confirmed
to be novel sequences not reported to date.

5 Example 3 Creation of recombinant baculovirus capable of
 yielding virus-like particles

 (1) Construction of transfer vectors

 The plasmids with the structural gene regions incorporated
therein, which had been obtained in Example 1(3), were digested
10 by a restriction endonuclease which does not cleave the
structural gene regions. Subsequent to separation by agarose
gel electrophoresis, the structural gene regions were recovered
by "SuprecTM01" (TAKARA). The recovered gene fragments were
incorporated in baculovirus transfer vectors pVL1393 (product
15 of Invitrogen Corporation), which had been digested by the same
restriction endonuclease, to prepare transfer vectors.

 (2) Creation of recombinant baculoviruses

 Baculovirus DNA (0.5 μ g; "Baculo-Gold") and one of the
transfer vectors (1 μ g) obtained in (1) were dissolved in
20 distilled water (8 μ L). The resulting solution was mixed with
a two-fold dilution of lipofectin (equivalent amount), and the
thus-obtained mixture was left over at room temperature for 15
minutes. After Sf9 cells (1×10^5 cells) suspended in an insect
cell culture medium, "Ex-cell 400", were adsorbed at 26.5°C for
25 30 minutes in a plastic Petri dish (diameter: 3.5 cm), a mixture

of the transfer vector and "Baculo-Gold" was added dropwise to the cells, followed by incubation at 26.5°C. 24 Hours later, the culture medium was replaced by a "TC100" (product of GIBCO BRL Life Technologies; hereinafter referred to as "TC100") which
5 contained 10% fetal bovine serum and 2% BTB (products of GIBCO BRL Life Technologies), and incubation was continued further.

(3) Purification of recombinant baculoviruses

After each recombinant baculovirus obtained in (2) was incubated for 5 days, the culture supernatant was diluted tenfold
10 with an insect cell culture medium such as TC100. A 0.1-mL aliquot of the diluted supernatant was taken, and inoculated to 3×10^6 Sf9 cells cultured in a plastic Petri dish of 3.5 cm in diameter. Subsequent to adsorption at 26.5°C for 60 minutes, TC100 culture medium (2 mL) which contained 1% of Agarose ME
15 (low melting-point agarose) was overlayed, followed by incubation at 26.5°C. On the 4th day after the initiation of the incubation, TC100 (1 mL) which contained 0.005% of neutral red was further overlayed, followed by incubation at 26.5°C. On the following day, the formed plaques were scraped off with
20 a microtip. and were suspended in TC100 culture medium.

(4) Production of recombinant baculovirus seeds and measurement of their infective potencies

Each suspension obtained in (3) was inoculated to 1×10^7 Sf9 cells. Subsequent to adsorption at 26.5°C for 60 minutes, TC100 was added, followed by incubation at 26.5°C for 3 to 4
25

days. The culture was centrifuged at 2,500 rpm for 10 minutes at 4°C, and the culture supernatant was collected. The collected culture supernatant was inoculated to 1×10^7 Sf9 cells.

Subsequent to adsorption at 26.5°C for 60 minutes, TC100 was added, followed by incubation at 26.5°C for 3 to 4 days.

Next, the culture supernatant was inoculated to 3×10^7 Sf9 cells cultured in a plastic Petri dish of 3.5 cm in diameter. Subsequent to adsorption at 26.5°C for 60 minutes, TC100 culture medium (2 mL) which contained 1% of Agarose ME (low melting-point agarose) was overlayed, followed by incubation at 26.5°C. On the 4th day after the initiation of the incubation, TC100 (1 mL) which contained 0.005% of neutral red was then overlayed, followed by incubation at 26.5°C. On the following day, the formed plaques were measured to calculate the infective potency of the recombinant baculovirus. This was recorded as the infective potency of the recombinant baculovirus.

Example 4 Creation of virus-like particles

(1) Expression of structural proteins by using recombinant baculoviruses

Each recombinant baculovirus was infected at M.O.I.s (Multiplicities of infection) of 1 to 10 to Sf9 insect cells. Upon infection, a suspension of the recombinant baculovirus was added dropwise to the cells, and the recombinant baculovirus was subjected to adsorption for about 60 minutes or so with gentle shaking. After that, TC100 was added as an insect cell culture

medium, followed by incubation at 26.5°C for 5 to 6 days.

(2) Identification of expressed proteins

The culture supernatant of each recombinant virus infection was periodically sampled. After having been resolved
5 by SDS-PAGE, the protein was detected by Coomassie blue staining, and by an expected molecular weight, the validity of the expressed protein was confirmation. Further, subsequent to resolving the protein by SDS-PAGE, the protein was transferred onto a nitrocellulose membrane, and by the Western blotting technique,
10 the expressed protein was then identified with a convalescent serum of the SRSV.

(3) Purification and recovery of virus-like particles

The recombinant baculovirus seeds were infected at M.O.I.s of from 1 to 10. Subsequent to adsorption for about 60 minutes,
15 "Ex-cell 400" was added, followed by incubation at 26.5°C for 3 days. A protease inhibitor, for example, pepstatin A or a leupeptin, was then added to the culture to a final concentration of 1 mM, followed by further incubation for 2 to 3 days.

Subsequent to the incubation, the culture was
20 centrifuged at 2,500 rpm for 10 minutes at 4°C to collect the culture supernatant. The collected culture was centrifuged at 10,000 rpm for 30 minutes to eliminate the recombinant baculovirus. The supernatant was centrifuged at 25,000 rpm for 4 hours on a "Beckmann SW28 Rotor" to have virus-like particles
25 precipitated. Then, the centrifuge tube from which the

supernatant had been discarded was held upside down to complete eliminate the supernatant. After that, Grace buffer or PBS(-) (0.5 mL) with the protease inhibitor added therein was added to the centrifuge tube, and the centrifuge was allowed to stand
5 overnight at 4°C.

After the standing, the virus-like particles were suspended in the protease-inhibitor-containing Grace buffer which had been added, and were recovered. To the recovered virus-like particles, protease-inhibitor-containing Grace
10 buffer or PBS(-) with CsCl (3.8 g) added therein was added to give 13 mL. The resulting mixture was ultracentrifuged at 16°C and 35,000 rpm for 24 to 48 hours. Subsequent to the ultracentrifugation, a pale band in which virus-like particles gathered was collected. After 5-fold dilution with
15 protease-inhibitor-containing Grace buffer, the resultant suspension was ultracentrifuged at 45,000 rpm for 3 hours on a "Beckmann TL100.3 Rotor" to have the virus-like particles precipitated.

The precipitated virus-like particles were solubilized
20 with Grace buffer or PBS(-) to which the protease inhibitor had been added. Protease-inhibitor-containing Grace buffer solutions which contained 10% to 50% of sucrose were prepared in a 4PA tube, into which the solubilized solution of the virus-like particles was overlayed, followed by sucrose
25 density-gradient centrifugation at 35,000 rpm for 4 hours at

4°C. Subsequent to the centrifugation, a pale band of virus-like particles was collected as purified SRSV virus-like particles in a 1-mL syringe fitted with a 26G needle.

The purified SRSV virus-like particles was diluted with Grace buffer as needed, and the quantity of protein was measured by the Bradford method.

The purified SRSV virus-like particles were subjected to negative staining with uranyl acetate, and were then examined by an electron microscope to ascertain whether or not virus-like particles had been formed (FIGS. 2 to 12).

Example 5 Preparation of Immune Antibodies and Labeled by Use of Antibodies Virus-like Particles

(1) Preparation of immune antibodies against virus-like particles

A phosphate buffer (pH 7.2, 1 mL) - which contained the purified SRSV virus-like particles (500 ig) obtained from one of the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, the Hu/NLV/Chiba 407/1987/JP strain, the Hu/NLV/Narita 104/1997/JP strain, Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain - and the Freund's incomplete adjuvant (1 mL) were mixed, and then immunized to a New Zealand white rabbit

(3 kg) in a manner known *per se* in the art. Three weeks later, the rabbit was immunized further with a mixture of a phosphate buffer (pH 7.2, 1 mL), which contained the SRSV virus-like particles (0.25 μ g), and the Freund's incomplete adjuvant (1 mL) (booster dose). Additional 3 weeks later, immunization was conducted as in the booster dose, and about 7 to 10 days after the additional booster dose, exsanguination was conducted, and the serum component was separated.

After the separated and purified serum was subjected to ammonium sulfate fractionation, the relevant fraction was dialyzed overnight at 4°C against 50 mM Tris-HCl (pH 7.6). The inner dialyzate was then subjected to DEAE Sepharose chromatography which had been equilibrated with 50 mM Tris-HCl (pH 7.6). Under monitoring at an UV wavelength of 280 nm, an O.D. peak was collected to obtain an DEAE-purified IgG antibody (anti-SRSV antibody) against the virus-like particles.

(2) Preparation of labeled antibodies

Each anti-SRSV antibody was labeled with POD by an improved periodic acid technique ["Koso Men-eki Sokuteiho (Enzyme Immunoassay)", 2, 91, 1982]. Described specifically, POD was dissolved at 4 mg/mL in distilled water and 0.1 M sodium periodate (0.2 mL) was added, followed by a reaction at room temperature for about 20 minutes. The reaction mixture was then dialyzed overnight against 1 mM sodium acetate buffer (pH 4.0). Subsequent to the dialysis, 0.2 M sodium carbonate buffer (pH

9.5, 0.02 mL) was added to adjust the pH to 9.5, and at the same time, the anti-SRSV antibody (8 mg) was added.

After having been allowed to react at room temperature for 2 hours, 4 mg/mL sodium borohydroxide (0.1 mL) was added, followed by a reaction at 4°C for about 2 hours. After the reaction, gel filtration was conducted with "Sephacryl S-200" while using 10 mM phosphate buffer. Under monitoring at an UV wavelength of 280 nm, a POD-labeled anti-SRSV antibody fraction was collected.

10 (3) Preparation of a solid-phase anti-SRSV antibody microplate

The anti-SRSV antibodies were separately diluted with a carbonate buffer (pH 9.5) to a concentration of from 0.5 to 10 µg/mL and then poured at 100 µL/well into a polystyrene flat-bottom microplate (manufactured by Nunc). The microplate was then allowed to stand overnight 4°C. After standing for 18 hours or longer, the microplate was washed 3 to 4 times at 200 µL/well with PBS which contained "Tween 20" at a final concentration of 0.05%. 10 mM PBS (pH 7.2) - which contained bovine serum albumin (BSA) and "Tween 20" at final concentrations of 0.5% and 0.05%, respectively - was then added at 200 µL/well. The microplate was allowed to stand overnight 4°C to obtain a solid-phase anti-SRSV antibody microplate.

Example 6 Cross-reactivity

25 (1) Antigen detection ELISA

The purified SRSV virus-like particles of each group were diluted to 4 ng/mL to 0.04 ng/mL with a solution containing bovine serum albumin (BSA) and "Tween 20" at final concentrations of 0.2% and 0.05%, respectively, in a buffer (10 mM PBS, pH 7.2).

5 Then, the diluted emulsions of the virus-like particles (VLPs) were each added at 100 μ L/well to wells of the corresponding solid-phase anti-SRSV antibody microplate, followed by a reaction at room temperature for 60 minutes. After the reaction, the reaction mixtures in the wells were eliminated under suction.

10 10 mM PBS (pH 7.2) which contained "Tween 20" at a final concentration of 0.05% was added at 200 μ L/well to the wells, and was then eliminated under suction likewise. This procedure was repeated at least three times. After washing, the

15 POD-labeled anti-SRSV antibody of the corresponding serotype, which had been diluted 20000-fold with a buffer, was added at 100 μ L/well, followed by a reaction at room temperature for 60 minutes. Subsequent to washing, a TMB solution with hydrogen peroxide contained therein was added at 100 μ L/well, followed

20 by a reaction at room temperature for 30 minutes. After the reaction, 0.6 N sulfuric acid was added at 100 μ L/well, and the absorbance (450 nm/630 nm) of each well was measured by an ELISA autoreader. The results are shown in Table 2.

Table 2 Cross-reactivity between Serotypes

Purified VLP	VLP concentration (ng/mL)	Solid-phase antibody plate x POD (top: strain name, bottom: dilution of POD-labeled antibody)											
		124	258	407	645	104	809	754	1876	47	7k	10-25	
		20000	20000	20000	20000	20000	20000	20000	20000	20000	20000	20000	
124	4	1.430	0.018	0.013	0.016	0.013	0.007	0.007	0.008	0.010	0.019	0.009	
	0.4	0.192	0.011	0.010	0.011	0.014	0.007	0.007	0.008	0.011	0.018	0.009	
	0.04	0.030	0.011	0.011	0.011	0.013	0.007	0.007	0.009	0.012	0.018	0.009	
258	4	0.042	1.831	0.114	0.020	0.015	0.009	0.007	0.010	0.012	0.019	0.010	
	0.4	0.013	0.270	0.022	0.013	0.016	0.009	0.007	0.009	0.013	0.019	0.011	
	0.04	0.008	0.043	0.012	0.011	0.017	0.008	0.007	0.009	0.012	0.018	0.010	
407	4	0.084	0.045	0.974	0.010	0.015	0.007	0.007	0.009	0.011	0.018	0.009	
	0.4	0.016	0.012	0.134	0.010	0.013	0.007	0.008	0.009	0.011	0.018	0.009	
	0.04	0.009	0.010	0.025	0.011	0.014	0.007	0.007	0.008	0.011	0.019	0.009	
645	4	0.149	0.034	0.023	0.320	0.016	0.008	0.008	0.009	0.011	0.020	0.010	
	0.4	0.024	0.013	0.012	0.045	0.017	0.009	0.008	0.009	0.012	0.019	0.011	
	0.04	0.010	0.010	0.011	0.014	0.015	0.009	0.008	0.008	0.012	0.021	0.011	
104	4	0.007	0.009	0.009	0.010	0.708	0.007	0.015	0.025	0.017	0.031	0.009	
	0.4	0.010	0.009	0.009	0.010	0.094	0.008	0.008	0.011	0.013	0.020	0.009	
	0.04	0.009	0.009	0.010	0.011	0.024	0.008	0.007	0.009	0.012	0.020	0.009	
809	4	0.013	0.012	0.012	0.011	0.114	0.877	0.047	0.143	0.046	0.080	0.017	
	0.4	0.010	0.010	0.011	0.011	0.030	0.134	0.013	0.033	0.018	0.028	0.013	
	0.04	0.009	0.010	0.010	0.010	0.017	0.022	0.008	0.011	0.014	0.020	0.011	
754	4	0.008	0.011	0.009	0.010	0.038	0.008	0.286	0.068	0.025	0.027	0.013	
	0.4	0.008	0.009	0.010	0.011	0.017	0.008	0.038	0.015	0.013	0.020	0.010	
	0.04	0.009	0.009	0.011	0.011	0.016	0.008	0.011	0.010	0.012	0.020	0.009	
1876	4	0.010	0.012	0.011	0.011	0.026	0.009	0.013	0.728	0.023	0.025	0.012	
	0.4	0.009	0.014	0.010	0.011	0.017	0.009	0.008	0.089	0.015	0.021	0.013	
	0.04	0.011	0.010	0.010	0.012	0.016	0.010	0.007	0.017	0.014	0.019	0.011	
47	4	0.008	0.009	0.009	0.010	0.017	0.007	0.008	0.011	0.324	0.021	0.014	
	0.4	0.008	0.009	0.009	0.011	0.015	0.008	0.008	0.009	0.048	0.020	0.013	
	0.04	0.008	0.009	0.009	0.011	0.014	0.008	0.008	0.008	0.017	0.022	0.011	

Table 2 (Cont'd)

Purified VLP	VLP concentration (ng/mL)	Solid-phase antibody plate x POD (top: strain name, bottom: dilution of POD-labeled antibody)										
		124	258	407	645	104	809	754	1876	47	7k	10-25
7k	4	20000	20000	20000	20000	20000	20000	20000	20000	20000	20000	20000
	0.4	0.009	0.010	0.010	0.011	0.019	0.009	0.010	0.011	0.015	0.160	0.014
	0.04	0.009	0.011	0.010	0.011	0.016	0.008	0.008	0.008	0.015	0.035	0.016
10-25	4	0.011	0.010	0.010	0.011	0.017	0.009	0.008	0.009	0.014	0.022	0.015
	0.4	0.009	0.010	0.010	0.011	0.098	0.010	0.022	0.069	0.033	0.058	1.050
	0.04	0.007	0.009	0.010	0.011	0.026	0.009	0.009	0.020	0.018	0.026	0.163
Blank		0.009	0.009	0.009	0.012	0.016	0.009	0.007	0.011	0.015	0.023	0.029
		0.009	0.011	0.010	0.011	0.016	0.009	0.008	0.009	0.017	0.022	0.016

In the table, "645" indicates the Hu/NLV/Kashiwa 645/1999/JP strain, "124" the Hu/NLV/Seto 124/1989/JP strain, "258" the Hu/NLV/Funabashi 258/1996/JP strain, "407" the Hu/NLV/Chiba 407/1987/JP strain, "104" the Hu/NLV/Narita 104/1997/JP strain, "809" the Hu/NLV/Sanbu 809/1998/JP strain, "754" the Hu/NLV/Ichikawa 754/1998/JP strain, "1876" the Hu/NLV/Chitta 1876/1996/JP strain, "47" the Hu/NLV/Kashiwa 47/1997/JP strain, "7k" the Hu/NLV/Mie 7k/1994/JP strain, and "10-25" the Hu/NLV/Osaka 10-25/1999/JP strain.

As a result, no cross-reactivity was observed between viruses of the same genogroup, to say nothing of cross-reactivity between viruses of different Genogroups I and II. It was, therefore, confirmed that the serotypes of the 11 types of used virus strains were different from one another.

Test 1 Discrimination of SRSVs in Genogroup

The anti-SRSV antibodies against the SRSVs belonging to Genogroup I (the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, and the Hu/NLV/Chiba 407/1987/JP strain) were diluted with a carbonate buffer (pH 9.5) to a concentration of from 0.5 to 10 μ g/mL and were then mixed. The thus-obtained mixture was poured at 100 μ L/well into a polystyrene flat-bottom microplate (manufactured by Nunc). The microplate was allowed to stand overnight at 4°C. After standing for 18 hours or longer, the microplate was washed 3 to 4 times at 200 μ L/well with PBS which contained "Tween 20" at a final concentration of 0.05%.

10 mM PBS (pH 7.2) - which contained bovine serum albumin (BSA) and "Tween 20" at final concentrations of 0.5% and 0.05%, respectively - was then added at 200 μ L/well. The microplate was allowed to stand overnight at 4°C to obtain a microplate with the anti-SRSV-IgG antibodies against the respective serotypes of Genogroup I carried in a mixed solid-phase form (Type I plate).

Next, the anti-SRSV antibodies against the SRSVs belonging to Genogroup II (the Hu/NLV/Narita 104/1997/JP strain, the Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain) were similarly formed into a solid phase to obtain a Type II plate.

To feces (0.5 to 1.0 g) of each SRSV patient, PBS (9 mL) and "Daiflon" (1 mL) were added, followed by homogenization. The thus-prepared suspension was centrifuged under 19,000 g for 20 minutes, and the supernatant was collected and formed into a 10% fecal emulsion. The 10% fecal emulsion was diluted at 1:1 in volume with a buffer. The diluted emulsion was added at 100 μ L/well into wells of the Type I and Type II plates, and was allowed to react at room temperature for 60 minutes. After the reaction, the reaction mixtures in the wells were eliminated under suction. 10 mM PBS (pH 7.2) - which contained "Tween 20" at a final concentration of 0.05% - was added at 200 μ L/well to the wells, and was then eliminated under suction. This

procedure was performed at least three times. After the washing, the POD-labeled anti-SRSV antibodies of the respective serotypes, said antibodies having had been diluted 20,000-fold with a buffer, were added at 100 μ L/well, and were then reacted at room temperature for 60 minutes. After washing, a TMB solution with hydrogen peroxide contained therein was added at 100 μ L/well, and were then reacted at room temperature for 30 minutes. Subsequent to the reaction, 0.6 N sulfuric acid was added at 100 μ L/well, and the absorbance (450 nm/630 nm) of each well was measured by an ELISA autoreader.

As a result, it was found that among 15 fecal specimens from patients infected to SRSV of Genogroup I, 14 fecal specimens reacted only to the Type I plate and did not react to the Type II plate. Concerning 7 fecal specimens from patients infected to SRSV of Genogroup II, on the other hand, 6 fecal specimens did not react to the Type I plate but reacted only to Type II plate. It has, therefore, been confirmed that discrimination in genogroup is actually feasible.

Test 2 Discrimination of SRSVs in Serotype

The anti-SRSV antibodies against the SRSVs (the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, the Hu/NLV/Chiba 407/1987/JP strain, the Hu/NLV/Narita 104/1997/JP strain, the Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the

Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain) were each independently diluted with a carbonate buffer (pH 9.5) to a concentration of from 0.5 to 10 μ g/mL. The thus-obtained dilutions were poured at 100 μ L/well into a polystyrene flat-bottom microplate (manufactured by Nunc). The microplate was allowed to stand overnight at 4°C. After having been allowed to stand for 18 hours or longer, the microplate was washed 3 to 4 times at 200 μ L/well with PBS which contained "Tween 20" at a final concentration of 0.05%. 10 mM PBS (pH 7.2) - which contained bovine serum albumin (BSA) and "Tween 20" at final concentrations of 0.5% and 0.05%, respectively - was then added at 200 μ L/well. The microplate was allowed to stand overnight at 4°C to obtain a solid-phase anti-SRSV antibody microplate (serotype discrimination plate).

With respect to fecal specimens from SRSV patients, ELISA was conducted in a similar manner as in Test 1. The results are shown in Table 3.

Table 3 Clinical Test

Total number of specimens: 41

Serotype discriminated by invention kit	Number of detected specimen(s)
HU/NLV/Kashiwa 645/1999/JP	1
Hu/NLV/Seto 124/1989/JP	7
Hu/NLV/Funabashi 258/1996/JP	4
Hu/NLV/Chiba 407/1987/JP	1
HU/NLV/Narita 104/1997/JP	4
Hu/NLV/Sanbu 809/1998/JP	12
Hu/NLV/Ichikawa 754/1998/JP	2
Hu/NLV/Chitta 1876/1996/JP	3
Hu/NLV/Kashiwa 47/1997/JP	1
Hu/NLV/Mie 7k/1994/JP	1
Hu/NLV/Osaka 10-25/1999/JP	2
Total number of detected specimens	38 (93%)

As a result, it has been found that according to the SRSV
5 detection method of the present invention, SRSVs can be detected
with a probability as high as 93% and their serotypes can also
be discriminated.

Further, the serotypes discriminated by the kit of the
present invention were consistent with those ascertained by PCR
10 and an analysis of their base sequences (Table 4).

Table 4 Ascertainment of Serotypes

Total number of specimens: 38

Serotype discriminated by invention kit	Number of specimen(s) discriminated in serotype by PCR and analysis of base sequences
HU/NLV/Kashiwa 645/1999/JP 1 Specimens	1
Hu/NLV/Seto 124/1989/JP 7 Specimens	7
Hu/NLV/Funabashi 258/1996/JP 4 Specimens	4
Hu/NLV/Chiba 407/1987/JP 1 Specimen	1
HU/NLV/Narita 104/1997/JP 4 Specimens	4
Hu/NLV/Sanbu 809/1998/JP 12 Specimens	12
Hu/NLV/Ichikawa 754/1998/JP 2 Specimens	2
Hu/NLV/Chitta 1876/1996/JP 3 Specimens	3
Hu/NLV/Kashiwa 47/1997/JP 1 Specimen	1
Hu/NLV/Mie 7k/1994/JP 1 Specimen	1
Hu/NLV/Osaka 10-25/1999/JP 2 Specimens	2

The anti-SRSV antibodies against the SRSVs (the

5 Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, the Hu/NLV/Chiba 407/1987/JP strain, the Hu/NLV/Narita 104/1997/JP strain, the Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta

10 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain) were each independently diluted with a

carbonate buffer (pH 9.5) to a concentration of from 0.5 to 10
ig/mL. All the dilutions so obtained were mixed. As an
alternative, the anti-SRSV antibodies may be diluted after mixing
them together. Using the thus-diluted mixture of the anti-SRSV
5 antibodies, a solid-phase anti-SRSV antibody microplate was
produced likewise. With respect to 22 fecal specimens from
patients infected to SRSV, ELISA was conducted in a similar manner
as in Test 1. It was possible to detect SRSV in 20 specimens.

10 **Industrial Applicability**

According to the SRSV detection kit of the present
invention, it is possible to detect most of the SRSV-related
viruses discovered to date and also to discriminate their
serotypes and genogroups. When SRSV-related food poisoning
15 occurs, the SRSV detection kit of the present invention is,
therefore, useful for specifying an infection route, preventing
the infection from spreading, and performing an epidemiological
investigation.

41-1

SEQUENCE LISTING

<110> DENKA SEIKEN CO.,LTD
National Institute of Infectious Diseases

<120> Detection Kit for SRSV

<130> DK0001

<150> JP P1999-175928

<151> 1999-06-22

<160> 34

<170> PatentIn Ver. 2.1

<210> 1

<211> 545

<212> PRT

<213> Hu/NLV/Kashiwa 645/1999/JP

<400> 1

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 20          25          30

Met Glu Pro Val Ala Gly Ala Ala Thr Ala Ala Ala Thr Ala Gly Gln
 35          40          45

Val Asn Met Ile Asp Pro Trp Ile Met Asn Asn Tyr Val Gln Ala Pro
 50          55          60

Gln Gly Glu Phe Thr Ile Ser Pro Asn Asn Thr Pro Gly Asp Ile Leu
 65          70          75          80

Phe Asp Leu Gln Leu Gly Pro His Leu Asn Pro Phe Leu Ser His Leu
 85          90          95

Ala Gln Met Tyr Asn Gly Trp Val Gly Asn Met Lys Val Lys Val Leu
100          105          110

Leu Ala Gly Asn Ala Phe Thr Ala Gly Lys Ile Ile Ile Ser Cys Ile
115          120          125

Pro Pro Gly Phe Ala Ala Gln Asn Ile Ser Ile Ala Gln Ala Thr Met
130          135          140

Phe Pro His Val Ile Ala Asp Val Arg Val Leu Glu Pro Ile Glu Val
145          150          155          160

Pro Leu Glu Asp Val Arg Asn Val Leu Phe His Asn Asn Asp Asn Ala
165          170          175

Pro Thr Met Arg Leu Val Cys Met Leu Tyr Thr Pro Leu Arg Ala Ser
180          185          190

Gly Ser Ser Ser Gly Thr Asp Pro Phe Val Ile Ala Gly Arg Val Leu
195          200          205

Thr Cys Pro Ser Pro Asp Phe Ser Phe Leu Phe Leu Val Pro Pro Asn
210          215          220

Val Glu Gln Lys Thr Lys Pro Phe Ser Val Pro Asn Leu Pro Leu Asn
225          230          235          240

Thr Leu Ser Asn Ser Arg Val Pro Ser Leu Ile Lys Ser Met Met Val
245          250          255

Ser Arg Asp His Gly Gln Met Val Gln Phe Gln Asn Gly Arg Val Thr
260          265          270

Leu Asp Gly Gln Leu Gln Gly Thr Thr Pro Thr Ser Ala Ser Gln Leu
275          280          285

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Cys Lys Ile Arg Gly Ser Val Phe His Ala Asn Gly Gly Asn Gly Tyr
 290 295 300
 Asn Leu Thr Glu Leu Asp Gly Ser Pro Tyr His Ala Phe Glu Ser Pro
 305 310 315 320
 Ala Pro Ile Gly Phe Pro Asp Leu Gly Glu Cys Asp Trp His Met Glu
 325 330 335
 Ala Ser Pro Thr Thr Gln Phe Asn Thr Gly Asp Val Ile Lys Gln Ile
 340 345 350
 Asn Val Lys Gln Glu Ser Ala Phe Ala Pro His Leu Gly Thr Ile Gln
 355 360 365
 Ala Asp Gly Leu Ser Asp Val Ser Val Asn Thr Asn Met Ile Ala Lys
 370 375 380
 Leu Gly Trp Val Ser Pro Val Ser Asp Gly His Arg Gly Asp Val Asp
 385 390 395 400
 Pro Trp Val Ile Pro Arg Tyr Gly Ser Thr Leu Thr Glu Ala Ala Gln
 405 410 415
 Leu Ala Pro Pro Ile Tyr Pro Pro Gly Phe Gly Glu Ala Ile Val Phe
 420 425 430
 Phe Met Ser Asp Phe Pro Ile Ala His Gly Thr Asn Gly Leu Ser Val
 435 440 445
 Pro Cys Thr Ile Pro Gln Glu Phe Val Thr His Phe Val Asn Glu Gln
 450 455 460
 Ala Pro Thr Arg Gly Glu Ala Ala Leu Leu His Tyr Leu Asp Pro Asp
 465 470 475 480
 Thr His Arg Asn Leu Gly Glu Phe Lys Leu Tyr Pro Glu Gly Phe Met
 485 490 495
 Thr Cys Val Pro Asn Ser Ser Gly Thr Gly Pro Gln Thr Leu Pro Ile
 500 505 510
 Asn Gly Val Phe Val Phe Val Ser Trp Val Ser Arg Phe Tyr Gln Leu
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 Lys Pro Val Gly Thr Ala Gly Pro Ala Cys Arg Leu Gly Ile Arg Arg
 530 535 540
 Ser
 545

<210> 2

<211> 530

<212> PRT

<213> Hu/NLV/Seto 124/1989/JP

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 20 25 30
 Met Asp Pro Val Ala Gly Ser Ser Thr Ala Val Ala Thr Ala Gly Gln
 35 40 45
 Val Asn Pro Ile Asp Pro Trp Ile Ile Asn Asn Phe Val Gln Ala Pro
 50 55 60
 Gln Gly Glu Phe Thr Ile Ser Pro Asn Asn Thr Pro Gly Gly Val Leu
 65 70 75 80
 Phe Asp Leu Ser Leu Gly Pro His Leu Asn Pro Phe Leu Leu His Leu
 85 90 95

41-3

Ser	Gln	Met	Tyr	Asn	Gly	Trp	Val	Gly	Asn	Met	Arg	Val	Arg	Ile	Met
			100					105					110		
Leu	Ala	Gly	Asn	Ala	Phe	Thr	Ala	Gly	Lys	Ile	Ile	Val	Ser	Cys	Ile
		115					120					125			
Pro	Pro	Gly	Phe	Gly	Ser	His	Asn	Leu	Thr	Ile	Ala	Gln	Ala	Thr	Leu
	130					135					140				
Phe	Pro	His	Val	Ile	Ala	Asp	Val	Arg	Thr	Leu	Asp	Pro	Ile	Glu	Val
145					150					155					160
Pro	Leu	Glu	Asp	Val	Arg	Asn	Val	Leu	Phe	His	Asn	Asn	Asp	Arg	Asn
			165						170					175	
Gln	Gln	Thr	Met	Arg	Leu	Val	Cys	Met	Leu	Tyr	Thr	Pro	Leu	Arg	Thr
			180					185					190		
Gly	Gly	Gly	Thr	Gly	Asp	Ser	Phe	Val	Val	Ala	Gly	Arg	Val	Met	Thr
		195					200					205			
Cys	Pro	Ser	Pro	Asp	Phe	Asn	Phe	Leu	Phe	Leu	Val	Pro	Pro	Thr	Val
	210					215					220				
Glu	Gln	Lys	Thr	Arg	Pro	Phe	Thr	Leu	Pro	Asn	Leu	Pro	Leu	Ser	Ser
225					230					235					240
Leu	Ser	Asn	Ser	Arg	Ala	Pro	Leu	Pro	Ile	Ser	Gly	Met	Gly	Ile	Ser
				245					250					255	
Pro	Asp	Asn	Val	Gln	Ser	Val	Gln	Phe	Gln	Asn	Gly	Arg	Cys	Thr	Leu
			260					265					270		
Asp	Gly	Arg	Leu	Val	Gly	Thr	Thr	Pro	Val	Ser	Leu	Ser	His	Val	Ala
		275					280					285			
Lys	Ile	Arg	Gly	Thr	Ser	Asn	Gly	Thr	Val	Ile	Asn	Leu	Thr	Glu	Leu
	290					295					300				
Asp	Gly	Thr	Pro	Phe	His	Pro	Phe	Glu	Gly	Pro	Ala	Pro	Ile	Gly	Phe
305					310					315					320
Pro	Asp	Leu	Gly	Gly	Cys	Asp	Trp	His	Ile	Asn	Met	Thr	Gln	Phe	Gly
				325					330					335	
His	Ser	Ser	Gln	Thr	Gln	Tyr	Asp	Val	Asp	Thr	Thr	Pro	Asp	Thr	Phe
			340					345					350		
Val	Pro	His	Leu	Gly	Ser	Ile	Gln	Ala	Asn	Gly	Ile	Gly	Ser	Gly	Asn
		355					360					365			
Tyr	Ile	Gly	Val	Leu	Ser	Trp	Val	Ser	Pro	Pro	Ser	His	Pro	Ser	Gly
	370					375					380				
Ser	Gln	Val	Asp	Leu	Trp	Lys	Ile	Pro	Asn	Tyr	Gly	Ser	Ser	Ile	Thr
385					390					395					400
Glu	Ala	Thr	His	Leu	Ala	Pro	Ser	Val	Tyr	Pro	Pro	Gly	Phe	Gly	Glu
				405					410					415	
Val	Leu	Val	Phe	Phe	Met	Ser	Lys	Ile	Pro	Gly	Pro	Gly	Ala	Tyr	Ser
			420					425					430		
Leu	Pro	Cys	Leu	Leu	Pro	Gln	Glu	Tyr	Ile	Ser	His	Leu	Ala	Ser	Glu
		435					440					445			
Gln	Ala	Pro	Thr	Val	Gly	Glu	Ala	Ala	Leu	Leu	His	Tyr	Val	Asp	Pro
	450					455					460				
Asp	Thr	Gly	Arg	Thr	Leu	Gly	Glu	Phe	Lys	Ala	Tyr	Pro	Asp	Gly	Phe
465					470					475					480
Leu	Thr	Cys	Val	Pro	Asn	Gly	Ala	Ser	Ser	Gly	Pro	Gln	Gln	Leu	Pro
				485					490					495	
Ile	Asn	Gly	Val	Phe	Val	Phe	Val	Ser	Trp	Val	Ser	Arg	Phe	Tyr	Gln

41-4

500 505 510
 Leu Lys Pro Val Gly Thr Ala Ser Ser Ala Arg Gly Arg Leu Gly Leu
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 Arg Arg
 530

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 20 25 30
 Met Glu Pro Val Ala Gly Pro Thr Thr Ala Val Ala Thr Ala Gly Gln
 35 40 45
 Val Asn Met Ile Asp Pro Trp Ile Val Asn Asn Phe Val Gln Ser Pro
 50 55 60
 Gln Gly Glu Phe Thr Ile Ser Pro Asn Asn Thr Pro Gly Asp Ile Leu
 65 70 75 80
 Phe Asp Leu Gln Leu Gly Pro His Leu Asn Pro Phe Leu Ser His Leu
 85 90 95
 Ser Gln Met Tyr Asn Gly Trp Val Gly Asn Met Arg Val Arg Ile Leu
 100 105 110
 Leu Ala Gly Asn Ala Phe Ser Ala Gly Lys Ile Ile Val Cys Cys Val
 115 120 125
 Pro Pro Gly Phe Thr Ser Ser Ser Leu Thr Ile Ala Gln Ala Thr Leu
 130 135 140
 Phe Pro His Val Ile Ala Asp Val Arg Thr Leu Glu Pro Ile Glu Met
 145 150 155 160
 Pro Leu Glu Asp Val Arg Asn Val Leu Tyr His Thr Asn Asp Asn Gln
 165 170 175
 Pro Thr Met Arg Leu Val Cys Met Leu Tyr Thr Pro Leu Arg Thr Gly
 180 185 190
 Gly Gly Ser Gly Asn Ser Asp Ser Phe Val Val Ala Gly Arg Val Leu
 195 200 205
 Thr Ala Pro Ser Ser Asp Phe Ser Phe Leu Phe Leu Val Pro Pro Thr
 210 215 220
 Ile Glu Gln Lys Thr Arg Ala Phe Thr Val Pro Asn Ile Pro Leu Gln
 225 230 235 240
 Thr Leu Ser Asn Ser Arg Phe Pro Ser Leu Ile Gln Gly Met Ile Leu
 245 250 255
 Ser Pro Asp Ala Ser Gln Val Val Gln Phe Gln Asn Gly Arg Cys Leu
 260 265 270
 Ile Asp Gly Gln Leu Leu Gly Thr Thr Pro Ala Thr Ser Gly Gln Leu
 275 280 285
 Phe Arg Val Arg Gly Lys Ile Asn Gln Gly Ala Arg Thr Leu Asn Leu
 290 295 300
 Thr Glu Val Asp Gly Lys Pro Phe Met Ala Phe Asp Ser Pro Ala Pro
 305 310 315 320
 Val Gly Phe Pro Asp Phe Gly Lys Cys Asp Trp His Met Arg Ile Ser

41-5

325 330 335
 Lys Thr Pro Asn Asn Thr Ser Ser Gly Asp Pro Met Arg Ser Val Ser
 340 345 350
 Val Gln Thr Asn Val Gln Gly Phe Val Pro His Leu Gly Ser Ile Gln
 355 360 365
 Phe Asp Glu Val Phe Asn His Pro Thr Gly Asp Tyr Ile Gly Thr Ile
 370 375 380
 Glu Trp Ile Ser Gln Pro Ser Thr Pro Pro Gly Thr Asp Ile Asn Leu
 385 390 395 400
 Trp Glu Ile Pro Asp Tyr Gly Ser Ser Leu Ser Gln Ala Ala Asn Leu
 405 410 415
 Ala Pro Pro Val Phe Pro Pro Gly Phe Gly Glu Ala Leu Val Tyr Phe
 420 425 430
 Val Ser Ala Phe Pro Gly Pro Asn Asn Arg Ser Ala Pro Asn Asp Val
 435 440 445
 Pro Cys Leu Leu Pro Gln Glu Tyr Ile Thr His Phe Val Ser Glu Gln
 450 455 460
 Ala Pro Thr Met Gly Asp Ala Ala Leu Leu His Tyr Val Asp Pro Asp
 465 470 475 480
 Thr Asn Arg Asn Leu Gly Glu Phe Lys Leu Tyr Pro Gly Gly Tyr Leu
 485 490 495
 Thr Cys Val Pro Asn Gly Val Gly Ala Gly Pro Gln Gln Leu Pro Leu
 500 505 510
 Asn Gly Val Phe Leu Phe Val Ser Trp Val Ser Arg Phe Tyr Gln Leu
 515 520 525
 Lys Pro Val Gly Thr Ala Ser Thr Ala Arg Ser Arg Leu Gly Val Arg
 530 535 540
 Arg Ile
 545

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 <211> 544
 <212> PRT
 <213> Hu/NLV/Chiba 407/1987/JP

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 35 40 45
 Val Asn Leu Ile Asp Pro Trp Ile Ile Asn Asn Phe Val Gln Ala Pro
 50 55 60
 Gln Gly Glu Phe Thr Ile Ser Pro Asn Asn Thr Pro Gly Asp Val Leu
 65 70 75 80
 Phe Asp Leu Gln Leu Gly Pro His Leu Asn Pro Phe Leu Ser His Leu
 85 90 95
 Ser Gln Met Tyr Asn Gly Trp Val Gly Asn Met Arg Val Arg Val Val
 100 105 110
 Leu Ala Gly Asn Ala Phe Thr Ala Gly Lys Val Ile Ile Cys Cys Val
 115 120 125
 Pro Pro Gly Phe Gln Ser Arg Thr Leu Ser Ile Ala Gln Ala Thr Leu

41-6

130		135		140
Phe Pro His Val Ile Ala Asp Val Arg Thr Leu Asp Pro Val Glu Val				
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Pro Leu Glu Asp Val Arg Asn Val Leu Tyr His Asn Asn Asp Thr Gln				
	165		170	175
Pro Thr Met Arg Leu Leu Cys Met Leu Tyr Thr Pro Leu Arg Thr Gly				
	180		185	190
Gly Ala Ser Gly Gly Thr Asp Ser Phe Val Val Ala Gly Arg Val Leu				
	195	200		205
Thr Cys Pro Gly Pro Asp Phe Asn Phe Leu Phe Leu Val Pro Pro Thr				
	210	215		220
Val Glu Gln Lys Thr Arg Pro Phe Thr Val Pro Asn Ile Pro Leu Lys				
225		230		235
Tyr Leu Ser Asn Ser Arg Ile Pro Asn Pro Ile Glu Gly Met Ser Leu				
	245		250	255
Ser Pro Asp Gln Thr Gln Asn Val Gln Phe Gln Asn Gly Arg Cys Thr				
	260	265		270
Ile Asp Gly Gln Pro Leu Gly Thr Thr Pro Val Ser Val Ser Gln Leu				
	275	280		285
Cys Lys Phe Arg Gly Arg Ile Thr Ser Gly Gln Arg Val Leu Asn Leu				
	290	295		300
Thr Glu Leu Asp Gly Ser Pro Phe Met Ala Phe Ala Ala Pro Ala Pro				
305		310		315
Ala Gly Phe Pro Asp Leu Gly Ser Cys Asp Trp His Ile Glu Met Ser				
	325	330		335
Lys Ile Pro Asn Ser Ser Thr Gln Asn Asn Pro Ile Val Thr Asn Ser				
	340	345		350
Val Lys Pro Asn Ser Gln Gln Phe Val Pro His Leu Ser Ser Ile Thr				
	355	360		365
Leu Asp Glu Asn Val Ser Ser Gly Gly Asp Tyr Ile Gly Thr Ile Gln				
	370	375		380
Trp Thr Ser Pro Pro Ser Asp Ser Gly Gly Ala Asn Thr Asn Phe Trp				
385		390		395
Lys Ile Pro Asp Tyr Gly Ser Ser Leu Ala Glu Ala Ser Gln Leu Ala				
	405	410		415
Pro Ala Val Tyr Pro Pro Gly Phe Asn Glu Val Ile Val Tyr Phe Met				
	420	425		430
Ala Ser Ile Pro Gly Pro Asn Gln Ser Gly Ser Pro Asn Leu Val Pro				
	435	440		445
Cys Leu Leu Pro Gln Glu Tyr Ile Thr His Phe Ile Ser Glu Gln Ala				
	450	455		460
Pro Ile Gln Gly Glu Ala Ala Leu Leu His Tyr Val Asp Pro Asp Thr				
465		470		475
Asn Arg Asn Leu Gly Glu Phe Lys Leu Tyr Pro Gly Gly Tyr Leu Thr				
	485	490		495
Cys Val Pro Asn Ser Ser Ser Thr Gly Pro Gln Gln Leu Pro Leu Asp				
	500	505		510
Gly Val Phe Val Phe Ala Ser Trp Val Ser Arg Phe Tyr Gln Leu Lys				
	515	520		525
Pro Val Gly Thr Ala Gly Pro Ala Arg Gly Arg Leu Gly Val Arg Arg				
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41-7

<210> 5
 <211> 539
 <212> PRT
 <213> Hu/NLV/Narita 104/1997/JP

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Asn	Leu	Val	Pro	Glu	Val	Asn	Asn	Glu	Val	Met	Ala	Leu	Glu	Pro	Val
			20					25					30		
Val	Gly	Ala	Ala	Ile	Ala	Ala	Pro	Val	Ala	Gly	Gln	Gln	Asn	Val	Ile
		35					40					45			
Asp	Pro	Trp	Ile	Arg	Asn	Asn	Phe	Val	Gln	Ala	Pro	Gly	Gly	Glu	Phe
	50					55					60				
Thr	Val	Ser	Pro	Arg	Asn	Ala	Pro	Gly	Glu	Ile	Leu	Trp	Ser	Ala	Pro
	65				70					75					80
Leu	Gly	Pro	Asp	Leu	Asn	Pro	Tyr	Leu	Ser	His	Leu	Ala	Arg	Met	Tyr
				85					90					95	
Asn	Gly	Tyr	Ala	Gly	Gly	Phe	Glu	Val	Gln	Val	Ile	Leu	Ala	Gly	Asn
			100					105					110		
Ala	Phe	Thr	Ala	Gly	Lys	Ile	Ile	Phe	Ala	Ala	Val	Pro	Pro	Asn	Phe
		115					120					125			
Pro	Thr	Glu	Gly	Leu	Ser	Pro	Ser	Gln	Val	Thr	Met	Phe	Pro	His	Ile
		130				135					140				
Ile	Val	Asp	Val	Arg	Gln	Leu	Glu	Pro	Val	Leu	Ile	Pro	Leu	Pro	Asp
	145				150					155					160
Val	Arg	Asn	Asn	Phe	Tyr	His	Tyr	Asn	Gln	Ser	Asn	Asp	Ser	Thr	Ile
				165					170					175	
Lys	Leu	Ile	Ala	Met	Leu	Tyr	Thr	Pro	Leu	Arg	Ala	Asn	Asn	Ala	Gly
			180					185					190		
Asp	Asp	Val	Phe	Thr	Val	Ser	Cys	Arg	Val	Leu	Thr	Arg	Pro	Ser	Pro
		195					200					205			
Asp	Phe	Asp	Phe	Ile	Phe	Leu	Val	Pro	Pro	Thr	Val	Glu	Ser	Arg	Thr
	210					215					220				
Lys	Pro	Phe	Thr	Val	Pro	Ile	Leu	Thr	Val	Glu	Glu	Met	Ser	Asn	Ser
	225				230					235					240
Arg	Phe	Pro	Ile	Pro	Leu	Glu	Lys	Leu	Tyr	Thr	Gly	Pro	Ser	Ser	Ala
				245					250					255	
Phe	Val	Val	Gln	Pro	Gln	Asn	Gly	Arg	Cys	Thr	Thr	Asp	Gly	Val	Leu
			260					265					270		
Leu	Gly	Thr	Thr	Gln	Leu	Ser	Ala	Val	Asn	Ile	Cys	Thr	Phe	Arg	Gly
		275					280					285			
Asp	Val	Thr	His	Ile	Ala	Gly	Ser	His	Asp	Tyr	Thr	Met	Asn	Leu	Ala
	290					295					300				
Ser	Gln	Asn	Trp	Ser	Asn	Tyr	Asp	Pro	Thr	Glu	Glu	Ile	Pro	Ala	Pro
	305				310					315					320
Leu	Gly	Thr	Pro	Asp	Phe	Val	Gly	Lys	Ile	Gln	Gly	Met	Leu	Thr	Gln
				325					330					335	
Thr	Thr	Arg	Glu	Asp	Gly	Ser	Thr	Arg	Ala	His	Lys	Ala	Thr	Val	Ser
			340					345					350		

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Thr Gly Ser Val His Phe Thr Pro Lys Leu Gly Ser Val Gln Tyr Thr
 355 360 365
 Thr Asp Thr Asn Asn Asp Phe Gln Thr Gly Gln Asn Thr Lys Phe Thr
 370 375 380
 Pro Val Gly Val Ile Gln Asp Gly Asn Asn His Gln Asn Glu Pro Gln
 385 390 395 400
 Gln Trp Val Leu Pro Asn Tyr Ser Gly Arg Thr Gly His Asn Val His
 405 410 415
 Leu Ala Pro Ala Val Ala Pro Thr Phe Pro Gly Glu Gln Leu Leu Phe
 420 425 430
 Phe Arg Ser Thr Met Pro Gly Cys Ser Gly Tyr Pro Asn Met Asn Leu
 435 440 445
 Asp Cys Leu Leu Pro Gln Glu Trp Val Gln His Phe Cys Gln Glu Ala
 450 455 460
 Ala Pro Ala Gln Ser Asp Val Ala Leu Leu Arg Phe Val Asn Pro Asp
 465 470 475 480
 Thr Gly Arg Val Leu Phe Glu Cys Lys Leu His Lys Ser Gly Tyr Val
 485 490 495
 Thr Val Ala His Thr Gly Pro His Asp Leu Val Ile Pro Pro Asn Gly
 500 505 510
 Tyr Phe Arg Phe Asp Ser Trp Val Asn Gln Phe Tyr Thr Leu Ala Pro
 515 520 525
 Met Gly Asn Gly Ala Gly Arg Arg Arg Ala Leu
 530 535

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<211> 548

<212> PRT

<213> Hu/NLV/Sanbu 809/1998/JP

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 20 25 30
 Ala Gly Ala Ala Ile Ala Ala Pro Leu Thr Gly Gln Gln Asn Ile Ile
 35 40 45
 Asp Pro Trp Ile Met Asn Asn Phe Val Gln Ala Pro Gly Gly Glu Phe
 50 55 60
 Thr Val Ser Pro Arg Asn Ser Pro Gly Glu Val Leu Leu Asn Leu Glu
 65 70 75 80
 Leu Gly Pro Glu Ile Asn Pro Tyr Leu Ala His Leu Ala Arg Met Tyr
 85 90 95
 Asn Gly Tyr Ala Gly Gly Phe Glu Val Gln Val Val Leu Ala Gly Asn
 100 105 110
 Ala Phe Thr Ala Gly Lys Ile Ile Phe Ala Ala Ile Pro Pro Asn Phe
 115 120 125
 Pro Ile Asp Asn Leu Ser Ala Ala Gln Ile Thr Met Cys Pro His Val
 130 135 140
 Ile Val Asp Val Arg Gln Leu Glu Pro Val Asn Leu Pro Met Pro Asp
 145 150 155 160
 Val Arg Asn Asn Phe Phe His Tyr Asn Gln Gly Ser Asp Ser Arg Leu
 165 170 175

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Arg Leu Ile Ala Met Leu Tyr Thr Pro Leu Arg Ala Asn Asn Ser Gly
 180 185 190
 Asp Asp Val Phe Thr Val Ser Cys Arg Val Leu Thr Arg Pro Ser Pro
 195 200 205
 Asp Phe Ser Phe Asn Phe Leu Val Pro Pro Thr Val Glu Ser Lys Thr
 210 215 220
 Lys Pro Phe Thr Leu Pro Ile Leu Thr Ile Ser Glu Met Ser Asn Ser
 225 230 235 240
 Arg Phe Pro Val Pro Ile Glu Ser Leu His Thr Ser Pro Thr Glu Asn
 245 250 255
 Ile Val Val Gln Cys Gln Asn Gly Arg Val Thr Leu Asp Gly Glu Leu
 260 265 270
 Met Gly Thr Thr Gln Leu Leu Pro Ser Gln Ile Cys Ala Phe Arg Gly
 275 280 285
 Val Leu Thr Arg Ser Thr Ser Arg Ala Ser Asp Gln Ala Asp Thr Ala
 290 295 300
 Thr Pro Arg Leu Phe Asn Tyr Tyr Trp His Val Gln Leu Asp Asn Leu
 305 310 315 320
 Asn Gly Thr Pro Tyr Asp Pro Ala Glu Asp Ile Pro Gly Pro Leu Gly
 325 330 335
 Thr Pro Asp Phe Arg Gly Lys Val Phe Gly Val Ala Ser Gln Arg Asn
 340 345 350
 Leu Asp Ser Thr Thr Arg Ala His Glu Ala Lys Val Asp Thr Thr Ala
 355 360 365
 Gly Arg Phe Thr Pro Lys Leu Gly Ser Leu Glu Ile Ser Thr Asp Ser
 370 375 380
 Asp Asp Phe Asp Gln Asn Gln Pro Thr Lys Phe Thr Pro Val Gly Ile
 385 390 395 400
 Gly Val Asp Asn Glu Ala Glu Phe Gln Gln Trp Ser Leu Pro Asp Tyr
 405 410 415
 Ser Gly Gln Phe Thr His Asn Met Asn Leu Ala Pro Ala Val Ala Pro
 420 425 430
 Asn Phe Pro Gly Glu Gln Leu Leu Phe Phe Arg Ser Gln Leu Pro Ser
 435 440 445
 Ser Gly Gly Arg Ser Asn Gly Val Leu Asp Cys Leu Val Pro Gln Glu
 450 455 460
 Trp Val Gln His Phe Tyr Gln Glu Ser Ala Pro Ala Gln Thr Gln Val
 465 470 475 480
 Ala Leu Val Arg Tyr Val Asn Pro Asp Thr Gly Lys Val Leu Phe Glu
 485 490 495
 Ala Lys Leu His Lys Leu Gly Phe Met Thr Ile Ala Asn Asn Gly Asp
 500 505 510
 Ser Pro Ile Thr Val Pro Pro Asn Gly Tyr Phe Arg Phe Glu Ser Trp
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 Arg Arg Ile Gln
 545

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41-10

<212> PRT

<213> Hu/NLV/Ichikawa 754/1998/JP

<400> 7

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Met Lys Met Ala Ser Asn Asp Ala Thr Pro Ser Asn Asp Gly Ala Ala
 1          5          10          15

Gly Leu Val Pro Glu Ser Asn Asn Glu Ala Met Ala Leu Glu Pro Val
 20          25          30

Val Gly Ala Ser Leu Ala Ala Pro Val Thr Gly Gln Thr Asn Ile Ile
 35          40          45

Asp Pro Trp Ile Arg Thr Asn Phe Val Gln Ala Pro Asn Gly Glu Phe
 50          55          60

Thr Val Ser Pro Arg Asn Ser Pro Gly Glu Ile Leu Val Asn Leu Glu
 65          70          75          80

Leu Gly Pro Glu Leu Asn Pro Tyr Leu Ala His Leu Ala Arg Met Tyr
 85          90          95

Asn Gly Tyr Ala Gly Gly Met Glu Val Gln Val Met Leu Ala Gly Asn
100          105          110

Ala Phe Thr Ala Gly Lys Ile Ile Phe Ala Ala Val Pro Pro Tyr Phe
115          120          125

Pro Val Glu Asn Leu Ser Pro Ser Gln Ile Thr Met Phe Pro His Val
130          135          140

Ile Ile Asp Val Arg Thr Leu Glu Pro Val Leu Leu Pro Met Pro Asp
145          150          155          160

Val Arg Ser Thr Leu Phe His Phe Asn Gln Lys Asp Glu Pro Lys Met
165          170          175

Arg Leu Val Ala Met Leu Tyr Thr Pro Leu Arg Ser Asn Gly Ser Gly
180          185          190

Asp Asp Val Phe Thr Val Ser Cys Arg Ile Leu Thr Arg Pro Ser Pro
195          200          205

Glu Phe Asp Phe Thr Tyr Leu Val Pro Pro Thr Val Glu Ser Lys Thr
210          215          220

Lys Pro Phe Thr Leu Pro Val Leu Thr Leu Gly Glu Leu Ser Asn Ser
225          230          235          240

Arg Phe Pro Leu Ser Ile Asp Glu Met Val Thr Ser Pro Asn Glu Ser
245          250          255

Ile Val Val Gln Pro Gln Asn Gly Arg Val Thr Leu Asp Gly Glu Leu
260          265          270

Leu Gly Thr Thr Gln Leu Gln Ala Cys Asn Ile Cys Ser Ile Arg Gly
275          280          285

Lys Val Thr Gly Gln Val Pro Ser Glu Gln His Met Trp Asn Leu Glu
290          295          300

Ile Thr Asn Leu Asn Gly Thr Gln Phe Asp Pro Thr Asp Asp Val Pro
305          310          315          320

Ala Pro Leu Gly Val Pro Asp Phe Ala Gly Glu Val Phe Gly Val Leu
325          330          335

Ser Gln Arg Asn Arg Gly Glu Ser Asn Pro Ala Asn Arg Ala His Asp
340          345          350

Ala Val Val Ala Thr Tyr Ser Asp Lys Tyr Thr Pro Lys Leu Gly Leu
355          360          365

Val Gln Ile Gly Thr Trp Asn Thr Asn Asp Val Glu Asn Gln Pro Thr
370          375          380

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41-11

Lys Phe Thr Pro Ile Gly Leu Asn Glu Val Ala Asn Gly His Arg Phe
 385 390 395 400
 Glu Gln Trp Thr Leu Pro Arg Tyr Ser Gly Ala Leu Thr Leu Asn Met
 405 410 415
 Asn Leu Ala Pro Ala Val Ala Pro Leu Phe Pro Gly Glu Arg Leu Leu
 420 425 430
 Phe Phe Arg Ser Tyr Val Pro Leu Lys Gly Gly Phe Gly Asn Pro Ala
 435 440 445
 Ile Asp Cys Ser Val Pro Gln Glu Trp Val Gln His Phe Tyr Gln Glu
 450 455 460
 Ser Ala Pro Ser Leu Gly Asp Val Ala Leu Val Arg Tyr Val Asn Pro
 465 470 475 480
 Asp Thr Gly Arg Val Leu Phe Glu Ala Lys Leu His Lys Gly Gly Phe
 485 490 495
 Leu Thr Val Ser Ser Thr Ser Thr Gly Pro Val Val Val Pro Ala Asn
 500 505 510
 Gly Tyr Phe Lys Phe Asp Ser Trp Val Asn Gln Phe Tyr Ser Leu Ala
 515 520 525
 Pro Met Gly Thr Gly Asn Gly Arg Arg Arg Val Gln
 530 535 540

<210> 8
 <211> 535
 <212> PRT
 <213> Hu/NLV/Chitta 1876/1996/JP

<400> 8
 Met Lys Met Ala Ser Asn Asp Ala Ala Pro Ser Asn Asp Gly Ala Ala
 1 5 10 15
 Gly Leu Val Pro Glu Ala Asn Asn Glu Thr Met Ala Leu Glu Pro Val
 20 25 30
 Ala Gly Ala Ser Ile Ala Ala Pro Leu Thr Gly Gln Asn Asn Ile Ile
 35 40 45
 Asp Pro Trp Ile Arg Leu Asn Phe Val Gln Ala Pro Asn Gly Glu Phe
 50 55 60
 Thr Val Ser Pro Arg Asn Ser Pro Gly Glu Val Leu Leu Asn Leu Glu
 65 70 75 80
 Leu Gly Pro Glu Leu Asn Pro Tyr Leu Ala His Leu Ser Arg Met Tyr
 85 90 95
 Asn Gly Tyr Ala Gly Gly Val Glu Val Gln Val Leu Leu Ala Gly Asn
 100 105 110
 Ala Phe Thr Ala Gly Lys Leu Val Phe Ala Ala Val Pro Pro His Phe
 115 120 125
 Pro Leu Glu Asn Ile Ser Pro Gly Gln Ile Thr Met Phe Pro His Val
 130 135 140
 Ile Ile Asp Val Arg Thr Leu Glu Pro Val Leu Leu Pro Leu Pro Asp
 145 150 155 160
 Val Arg Asn Asn Phe Phe His Tyr Asn Gln Gln Asn Glu Pro Arg Met
 165 170 175
 Arg Leu Val Ala Met Leu Tyr Thr Pro Leu Arg Ser Asn Gly Ser Gly
 180 185 190
 Asp Asp Val Phe Thr Val Ser Cys Arg Val Leu Thr Arg Pro Ser Pro
 195 200 205

41-12

Asp Phe Asp Phe Asn Tyr Leu Val Pro Pro Thr Leu Glu Ser Lys Thr
 210 215 220
 Lys Pro Phe Thr Leu Pro Ile Leu Thr Ile Gly Glu Leu Thr Asn Ser
 225 230 235 240
 Arg Phe Pro Val Pro Ile Asp Glu Leu Tyr Thr Ser Pro Asn Glu Ser
 245 250 255
 Leu Val Val Gln Pro Gln Asn Gly Arg Cys Ala Leu Asp Gly Glu Leu
 260 265 270
 Gln Gly Thr Thr Gln Leu Leu Pro Thr Ala Ile Cys Ser Phe Arg Gly
 275 280 285
 Arg Ile Asn Gln Lys Val Ser Gly Glu Asn His Val Trp Asn Met Gln
 290 295 300
 Val Thr Asn Ile Asn Gly Thr Pro Phe Asp Pro Thr Gly Asp Val Pro
 305 310 315 320
 Ala Pro Leu Gly Thr Pro Asp Phe Ser Gly Lys Leu Phe Gly Val Leu
 325 330 335
 Ser Gln Arg Asp His Asp Asn Ala Cys Arg Ser His Asp Ala Val Ile
 340 345 350
 Ala Thr Asn Ser Ala Lys Phe Thr Pro Lys Leu Gly Ala Ile Gln Ile
 355 360 365
 Gly Thr Trp Glu Glu Asp Asp Val His Ile Asn Gln Pro Thr Lys Phe
 370 375 380
 Thr Pro Val Gly Leu Phe Glu Asn Glu Gly Phe Asn Gln Trp Thr Leu
 385 390 395 400
 Pro Asn Tyr Ser Gly Ala Leu Thr Leu Asn Met Gly Leu Ala Pro Pro
 405 410 415
 Val Ala Pro Thr Phe Pro Gly Glu Gln Ile Leu Phe Phe Arg Ser His
 420 425 430
 Ile Pro Leu Lys Gly Gly Val Ala Asp Pro Val Ile Asp Cys Leu Leu
 435 440 445
 Pro Gln Glu Trp Ile Gln His Leu Tyr Gln Glu Ser Ala Pro Ser Gln
 450 455 460
 Ser Asp Val Ala Leu Ile Arg Phe Thr Asn Pro Asp Thr Gly Arg Val
 465 470 475 480
 Leu Phe Glu Ala Lys Leu His Arg Ser Gly Tyr Ile Thr Val Ala Asn
 485 490 495
 Thr Gly Ser Arg Pro Ile Val Val Pro Ala Asn Gly Tyr Phe Arg Phe
 500 505 510
 Asp Thr Trp Val Asn Gln Phe Tyr Ser Leu Ala Pro Met Gly Thr Gly
 515 520 525
 Asn Gly Arg Arg Arg Val Gln
 530 535

<210> 9
 <211> 542
 <212> PRT
 <213> Hu/NLV/Kashiwa 47/1997/JP

<400> 9
 Met Lys Met Ala Ser Asn Asp Ala Ala Pro Ser Asn Asp Gly Ala Ala
 1 5 10 15
 Ser Leu Val Pro Glu Gly Ile Asn Glu Thr Met Pro Leu Glu Pro Val
 20 25 30

41-13

Ala Gly Ala Ser Ile Ala Ala Pro Val Ala Gly Gln Thr Asn Ile Ile
 35 40 45
 Asp Pro Trp Ile Arg Thr Asn Phe Val Gln Ala Pro Asn Gly Glu Phe
 50 55 60
 Thr Val Ser Pro Arg Asn Ser Pro Gly Glu Ile Leu Leu Asn Leu Glu
 65 70 75 80
 Leu Gly Pro Asp Leu Asn Pro Tyr Leu Ala His Leu Ser Arg Met Tyr
 85 90 95
 Asn Gly Tyr Ala Gly Gly Val Glu Val Gln Val Leu Leu Ala Gly Asn
 100 105 110
 Ala Phe Thr Ala Gly Lys Ile Leu Phe Ala Ala Ile Pro Pro Asn Phe
 115 120 125
 Leu Val Asp Met Ile Ser Pro Ala Gln Ile Thr Met Leu Pro His Leu
 130 135 140
 Ile Val Asp Val Arg Thr Leu Glu Pro Ile Met Thr Pro Leu Pro Asp
 145 150 155 160
 Val Arg Asn Val Phe Tyr His Phe Asn Asn Gln Pro Gln Pro Arg Met
 165 170 175
 Arg Leu Val Ala Met Leu Tyr Thr Pro Leu Arg Ser Asn Gly Ser Gly
 180 185 190
 Asp Asp Val Phe Thr Val Ser Cys Arg Val Leu Thr Arg Pro Thr Pro
 195 200 205
 Asp Phe Glu Phe Ile Tyr Leu Val Pro Pro Ser Val Glu Ser Lys Thr
 210 215 220
 Lys Pro Phe Thr Leu Pro Ile Leu Thr Ile Ser Glu Leu Thr Asn Ser
 225 230 235 240
 Arg Phe Pro Ile Pro Ile Glu Gln Leu Tyr Thr Ala Pro Asn Glu Thr
 245 250 255
 Asn Val Val Gln Cys Gln Asn Gly Arg Cys Thr Leu Asp Gly Glu Leu
 260 265 270
 Gln Gly Thr Thr Gln Leu Leu Ser Ser Ala Val Cys Phe Leu Gln Gly
 275 280 285
 Arg Thr Val Ala Asp Asn Gly Asp Asn Trp Asp Gln Asn Leu Leu Gln
 290 295 300
 Leu Thr Tyr Pro Asn Gly Ala Ser Tyr Asp Pro Thr Asp Glu Val Pro
 305 310 315 320
 Ala Pro Leu Gly Thr Gln Asp Phe Ser Gly Met Leu Tyr Gly Val Leu
 325 330 335
 Thr Gln Asp Asn Val Asn Val Ser Thr Gly Glu Ala Lys Asn Ala Lys
 340 345 350
 Gly Ile Tyr Ile Ser Thr Thr Ser Gly Lys Phe Thr Pro Lys Ile Gly
 355 360 365
 Ser Ile Gly Leu His Ser Ile Thr Glu His Val His Pro Asn Gln Gln
 370 375 380
 Ser Arg Phe Thr Pro Val Gly Val Ala Val Asp Glu Asn Thr Pro Phe
 385 390 395 400
 Gln Gln Trp Val Leu Pro His Tyr Ala Gly Ser Leu Ala Leu Asn Thr
 405 410 415
 Asn Leu Ala Pro Ala Val Ala Pro Thr Phe Pro Gly Glu Gln Leu Leu
 420 425 430
 Phe Phe Arg Ser Arg Val Pro Cys Val Gln Gly Leu Gln Gly Gln Asp

41-14

435		440		445
Ala Phe Ile Asp Cys Leu Leu Pro Gln Glu Trp Val Asn His Phe Tyr				
450		455		460
Gln Glu Ala Ala Pro Ser Gln Ala Asp Val Ala Leu Ile Arg Tyr Val				
465		470		475
Asn Pro Asp Thr Gly Arg Thr Leu Phe Glu Ala Lys Leu His Arg Ser				
		485		490
Gly Phe Ile Thr Val Ser His Thr Gly Ala Tyr Pro Leu Val Val Pro				
		500		505
Pro Asn Gly His Phe Arg Phe Asp Ser Trp Val Asn Gln Phe Tyr Ser				
		515		520
Leu Ala Pro Met Gly Thr Gly Asn Gly Arg Arg Arg Ile Gln				
530		535		540

<210> 10
 <211> 550
 <212> PRT
 <213> Hu/NLV/Mie 7k/1994/JP

<400> 10
 Met Lys Met Ala Ser Asn Asp Ala Ala Pro Ser Asn Asp Gly Ala Ala
 1 5 10 15

Asn Leu Val Pro Glu Ala Asn Asp Glu Val Met Ala Leu Glu Pro Val
 20 25 30

Val Gly Ala Ser Ile Ala Ala Pro Val Val Gly Gln Gln Asn Ile Ile
 35 40 45

Asp Pro Trp Ile Arg Glu Asn Phe Val Gln Ala Pro Gln Gly Glu Phe
 50 55 60

Thr Val Ser Pro Arg Asn Ser Pro Gly Glu Met Leu Leu Asn Leu Glu
 65 70 75 80

Leu Gly Pro Glu Leu Asn Pro Tyr Leu Ser His Leu Ser Arg Met Tyr
 85 90 95

Asn Gly Tyr Ala Gly Gly Met Gln Val Gln Val Val Leu Ala Gly Asn
 100 105 110

Ala Phe Thr Ala Gly Lys Ile Ile Phe Ala Ala Val Pro Pro His Phe
 115 120 125

Pro Val Glu Asn Ile Ser Ala Ala Gln Ile Thr Met Cys Pro His Val
 130 135 140

Ile Val Asp Val Arg Gln Leu Glu Pro Val Leu Leu Pro Leu Pro Asp
 145 150 155 160

Ile Arg Asn Arg Phe Phe His Tyr Asn Gln Glu Asn Thr Pro Arg Met
 165 170 175

Arg Leu Val Ala Met Leu Tyr Thr Pro Leu Arg Ala Asn Ser Gly Glu
 180 185 190

Asp Val Phe Thr Val Ser Cys Arg Val Leu Thr Arg Pro Ala Pro Asp
 195 200 205

Phe Glu Phe Thr Phe Leu Val Pro Pro Thr Val Glu Ser Lys Thr Lys
 210 215 220

Pro Phe Thr Leu Pro Ile Leu Thr Leu Gly Glu Leu Ser Asn Ser Arg
 225 230 235 240

Phe Pro Ala Ala Ile Asp Met Leu Tyr Thr Asp Pro Asn Glu Ser Ile
 245 250 255

Val Val Gln Pro Gln Asn Gly Arg Cys Thr Leu Asp Gly Thr Leu Gln

41-15

	260		265		270
Gly Thr Thr	Gln Leu Val Pro Thr	Gln Ile Cys Ala Phe Arg Gly Thr			
	275	280	285		
Leu Ile Ser	Gln Thr Ala Arg Ala Ala Asp Ser Thr Asp Ser Pro Gln				
	290	295	300		
Arg Ala Arg	Asn His Pro Leu His Val Gln Val Lys Asn Leu Asp Gly				
	305	310	315	320	
Thr Gln Tyr	Asp Pro Thr Asp Asp Ile Pro Ala Val Leu Gly Ala Ile				
	325	330	335		
Asp Phe Lys	Gly Thr Val Phe Gly Val Ala Ser Gln Arg Asp Val Ser				
	340	345	350		
Gly Gln Gln	Glu Gln Gly His Tyr Ala Thr Arg Ala His Glu Ala His				
	355	360	365		
Ile Asp Thr	Thr Asp Pro Lys Tyr Ala Pro Lys Leu Gly Thr Ile Leu				
	370	375	380		
Ile Lys Ser	Gly Ser Asp Asp Phe Asn Thr Asn Gln Pro Ile Arg Phe				
	385	390	395	400	
Thr Pro Val	Gly Met Gly Asp Asn Asn Trp Arg Gln Trp Glu Leu Pro				
	405	410	415		
Asp Tyr Ser	Gly Arg Leu Thr Leu Asn Met Asn Leu Ala Pro Ala Val				
	420	425	430		
Ser Pro Ser	Phe Pro Gly Glu Arg Ile Leu Phe Phe Arg Ser Ile Val				
	435	440	445		
Pro Ser Ala	Gly Gly Tyr Gly Ser Gly Tyr Ile Asp Cys Leu Ile Pro				
	450	455	460		
Gln Glu Trp	Val Gln His Phe Tyr Gln Glu Ala Ala Pro Ser Gln Ser				
	465	470	475	480	
Ala Val Ala	Leu Val Arg Tyr Val Asn Pro Asp Thr Gly Arg Asn Ile				
	485	490	495		
Phe Glu Ala	Lys Leu His Arg Glu Gly Phe Leu Thr Val Ala Asn Cys				
	500	505	510		
Gly Asn Asn	Pro Ile Val Val Pro Pro Asn Gly Tyr Phe Arg Phe Glu				
	515	520	525		
Ala Trp Gly	Asn Gln Phe Tyr Thr Leu Ala Pro Met Gly Ser Gly Gln				
	530	535	540		
Gly Arg Arg	Arg Ala Gln				
	545	550			

<210> 11

<211> 541

<212> PRT

<213> Hu/NLV/Osaka 10-25/1999/JP

<400> 11

Met Lys Met	Ala Ser Asn Asp Ala Ala Pro Ser Ser Asp Gly Ala Ala
1	5 10 15
Gly Leu Val	Pro Glu Ile Asn Asn Glu Val Met Pro Leu Glu Pro Val
	20 25 30
Ala Gly Ala	Ser Leu Ala Thr Pro Val Val Gly Gln Gln Asn Ile Ile
	35 40 45
Asp Pro Trp	Ile Arg Asn Asn Phe Val Gln Ala Pro Ala Gly Glu Phe
	50 55 60
Thr Val Ser	Pro Arg Asn Ser Pro Gly Glu Ile Leu Leu Asp Leu Glu

41-16

65		70		75		80
Leu Gly Pro Asp	Leu Asn Pro Tyr	Leu Ala His	Leu Ala Arg	Met Tyr		
	85		90		95	
Asn Gly His	Ala Gly Gly Met	Glu Val Gln	Ile Val Leu	Ala Gly Asn		
	100		105		110	
Ala Phe Thr	Ala Gly Lys	Ile Ile Phe	Ala Ala Ile	Pro Pro Gly	Phe	
	115		120		125	
Pro Tyr Glu	Asn Leu Ser	Pro Ser Gln	Ile Thr Met	Cys Pro His	Val	
	130		135		140	
Ile Ile Asp	Val Arg Gln	Leu Glu Pro	Phe Leu Leu	Pro Met Pro	Asp	
	145		150		155	160
Ile Trp Asn	Asn Phe Phe	His Tyr Asn	Gln Gly Asn	Asp Pro Lys	Leu	
	165		170		175	
Arg Leu Val	Ala Met Leu	Tyr Thr Pro	Leu Arg Ala	Asn Asn Ser	Gly	
	180		185		190	
Asp Asp Val	Phe Thr Val	Ser Cys Arg	Val Leu Thr	Lys Pro Ser	Pro	
	195		200		205	
Asp Phe Glu	Phe Thr Phe	Leu Val Pro	Pro Thr Val	Glu Ser Lys	Thr	
	210		215		220	
Lys Gln Phe	Ala Leu Pro	Ile Leu Lys	Ile Ser Glu	Met Thr Asn	Ser	
	225		230		235	240
Arg Phe Pro	Val Pro Val	Asp Val Met	Tyr Thr Ala	Arg Asn Glu	Asn	
	245		250		255	
Gln Val Val	Gln Pro Gln	Asn Gly Arg	Val Thr Leu	Asp Gly Glu	Leu	
	260		265		270	
Leu Gly Thr	Thr Pro Leu	Leu Ala Val	Asn Ile Cys	Lys Phe Lys	Gly	
	275		280		285	
Glu Val Ile	Ala Lys Asn	Gly Asp Val	Arg Ser Tyr	Arg Met Asp	Met	
	290		295		300	
Glu Ile Thr	Asn Thr Asp	Gly Thr Pro	Ile Asp Pro	Thr Glu Asp	Thr	
	305		310		315	320
Pro Gly Pro	Ile Gly Ser	Pro Asp Phe	Gln Gly Ile	Leu Phe Gly	Val	
	325		330		335	
Ala Ser Gln	Arg Asn Lys	Asn Glu Gln	Asn Pro Ala	Thr Arg Ala	His	
	340		345		350	
Glu Ala Ile	Ile Asn Thr	Gly Gly Asp	His Leu Cys	Pro Gln Ile	Ser	
	355		360		365	
Ser Ser Glu	Ile Tyr Leu	Thr Ser Pro	Asn Ile Leu	Arg Cys Thr	Asn	
	370		375		380	
Pro Gln Pro	Leu Pro Gln	Ser Gly Leu	Arg Gly Thr	Ile Leu Ile	Arg	
	385		390		395	400
Ser Asp Asn	Gly His Cys	His Asp Met	Val Gly Thr	Ser Pro Thr	Thr	
	405		410		415	
Pro Thr Trp	Pro Gln Gln	Trp Arg Arg	Cys Ser Arg	Gly Ser Asn	Cys	
	420		425		430	
Cys Ser Ser	Gly His Arg	Tyr Pro Val	Pro Val Val	Met Asn Arg	Val	
	435		440		445	
Thr Trp Ile	Val Leu Ser	His Lys Ser	Gly Phe Ser	Thr Ser Thr	Arg	
	450		455		460	
Lys Leu Pro	Gln Leu Asn	Leu Arg Trp	Pro Leu Ile	Arg Phe Ile	Asn	
	465		470		475	480

41-17

Pro Asp Thr Gly Arg Val Leu Phe Glu Ala Arg Leu His Lys Gln Gly
485 490 495

Phe Ile Thr Val Ala His Thr Gly Asp Asn Pro Ile Val Met Pro Pro
500 505 510

Asn Gly Tyr Phe Arg Phe Glu Ala Trp Val Asn Gln Phe Tyr Ser Leu
515 520 525

Ala Pro Val Gly Thr Gly Lys Gly Arg Arg Arg Val Gln
530 535 540

<210> 12
<211> 1638
<212> DNA
<213> Hu/NLV/Kashiwa 645/1999/JP

<400> 12
atgatgatgg cgtctaagga cgccccaaca aacatggatg gcaccagtgg tgccggccag 60
ctggtaccag aggcaaatac agctgagcct atatcaatgg agcctgtggc tggggcagca 120
acagctgccg caaccgctgg ccaagttaat atgattgacc cctggataat gaataattat 180
gtgcaagccc ctcaaggtga atttaccata tcgcctaata acacaccagg tgatattttg 240
tttgatctac aattaggccc tcatctcaat cctttcttat cccatttggc ccaaattgtat 300
aacggttggg ttggcaatat gaaagtgaag gtcctattgg ctggtaatgc cttcacggct 360
ggtaaaataa tcattagttg cataccccct ggctttgctg cgaaaacat ttctatcgct 420
caggccacaa tgttccccca cgttatagct gatgttaggg ttttggaacc tattgaggtg 480
ccattggaag atgtgaggaa tgtgctgttc cataacaatg acaacgcacc aaccatgagg 540
ttggtgtgca tgctctacac ccccttgcca gccagtggta gctcatctgg aactgaccct 600
tttgtgattg ctgggcgtgt tctgacatgc ccaagccctg acttttagctt cttattcttg 660
gttcccccca atgtagagca aaagactaaa ccttttagtg tcccaaactt tccactgaat 720
accctttcaa attcaagagt cctttctcta attaaatcaa tgatgggtatc cagagaccat 780
gggcagatgg ttcagtttca aaacggtagg gtcaccctgg atgggcaact gcaaggcacc 840
acgcccacat cagctagcca gctgtgcaaa atcagaggca gtgtcttcca tgctaattgg 900
gggaatggat ataacctaac tgaattggat gggagcccat accatgcttt tgagagccct 960
gcgccaatag ggtttcctga tctaggtgaa tgtgattggc acatggaggc ctcccctacc 1020
acccaattca atactggtga tgttataaaa caaattaatg tcaaacaaga atcagcattt 1080
gctccccacc ttggtaccat acaagcagat ggcctgagtg atgtgagtg caacactaac 1140
atgatagcca aattgggatg ggtgtcacc gtcagtgatg gacatagagg agatgtcgat 1200
ccgtgggtca ttccacgata tgggtcgact ttgaccgagg ccgcccatt agcccccca 1260
atatatcccc caggtttttg tgaggccatt gtgtttttca tgtcagattt tctatagcc 1320
catggtacca atggcttgag tgtgccttgc accatacccc aagaatttgt caccatttt 1380
gtcaatgaac agggccctac tagaggggaa gcagccctac tgcattattt agaccctgat 1440
acccatagaa atcttggtga gtttaaatta taccctgagg ggttcacgac gtgtgtgcct 1500
aattccagtg gcactggtcc acaaaccctc ccaatcaatg gtgtttttgt ttttgtgtcc 1560
tgggtttcca gattctatca gttaaagcct gtgggaacag ccggcccggc ttgtaggctt 1620
ggcatcagaa gatcataa 1638

<210> 13
<211> 1593
<212> DNA
<213> Hu/NLV/Seto 124/1989/JP

<400> 13
atgatgatgg cgtctaagga cgctacgtca agcgtggatg gcgccagtgg cgctgggtcag 60
ttggtaccgg aggttaatgc ttctgaccct cttgcaatgg atcctgtggc ggggttcttca 120
acagcagttg caactgctgg gcaagttaac cctattgacc cttggataat caataacttt 180
gtgcaggctc cccaaggtga atttactatt tctccaaata atacccccgg tgggtgtttg 240
tttgatttga gtctaggccc tcatcttaat cctttcttgt tacatttgtc acaaattgtat 300
aatggctggg ttggcaacat gagagttagg attatgctgg ctggtaatgc atttactgca 360
ggcaaaatta tagtttcttg catacctcct ggctttggct ccataatct tactatagca 420
caagcaactc tcttcccgca tgtgattgct gatgttagga ctttagacct aattgaagta 480
cccttggaag atgtaaggaa tgttctcttt cataataatg atagaaatca acaaaccatg 540
cgccttgtgt gtatgcttta tccccctc cgcactgggt gcggtacagg tgattctttt 600
gtggttgcag ggcgagtcac gacttgtcct agccccgatt tcaatttctt gttcttgggt 660
cctcccacag tggacagaa gactaggcct ttcaccctcc caaatttacc gctgagttct 720
ttgtcaaat cactgtctcc tcttccaaat agtggcatgg gtatttctcc agacaatgtt 780
cagagtgtgc agtttcaaaa tggccgatgt accttagacg ggcgtcttgt tggtagcacc 840
ccagtttccc tctcccacgt tgctaagata aggggcactt ctaatggtag tgtgatcaat 900
ctcaccgaat tggatggcac ccccttccac ccttttgaag gccctgcccc tattggtttt 960
ccagatcttg gtggctgtga ttggcatatt aatatgacac aatttgggca ttccagtcag 1020
actcaatatg atgtagatac ccccccgac accttcgtcc ctcaattagg ttcaatccag 1080
gcgaatggca ttggtagtgg caactatatt ggtgttctta gctgggtctc cccccatca 1140
catccatctg gctctcaagt tgatctctgg aagatcccca actatgggtc tagcatcaca 1200

41-18

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gaggcaaccc atctagctcc ctctgtctat cctcctggct ttggagaggt gttagtcttt 1260
ttcatgtcaa agatacctgg tcctgggtgt tatagtctgc cctgtttact gccacaagaa 1320
tatatctcac acctcgcaag tgaacaagcc cccactgttg gtgaggccgc cttgctccac 1380
tatgttgacc ctgacacggg cgggactctt ggggagttta aggcttacc tgatggtttc 1440
ctaacctgtg tccctaacgg gccagctcg gggccacaac aactaccaat caatggagtc 1500
tttgtctttg tttcatgggt gtccagattt tatcagttaa agcctgtggg aactgccagt 1560
tcggcaagag gtaggcttgg tttgcgccga taa 1593

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<210> 14
 <211> 1641
 <212> DNA
 <213> Hu/NLV/Funabashi 258/1996/JP

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<400> 14
atgatgatgg cgtctaagga cgccccctcaa agcgcctgatg gcgcaagcgg cgcagggtcaa 60
ctgggtgccgg aggttaatac agctgacccc ttacccatgg aaccctgtggc tgggccaaca 120
acagcccgtag ccactgctgg gcaagttaat atgattgatc cctggattgt taataatttt 180
gtccagtcac cacaaggtga gtttacaatt tcccctaata atacccccgg tgatatattt 240
tttgatttac aattagggtcc acatctaaac cttttcttgt cacatctgtc ccaaattgtat 300
aatggctggg ttggaaacat gagagttagg attctccttg ctgggaatgc attctcagct 360
ggaaagatta tagtttggtg tgtccccctt ggctttacat ctctctctct caccatagct 420
caggctacat tgtttcccca tgtgattgct gatgtgagaa cccttgaacc aatagaaatg 480
cccctcgagg atgtacgcaa tgtcctctat cacaccaatg ataatacaac aacaatgcgg 540
ttgggtgtgta tgctgtacac gccgctccgc actgggtgggg ggtctggtaa ttctgattct 600
tttgtgggtg ctggcagggg gctcacggcc cctagtagcg acttcagttt cttgttctct 660
gtcccgccta ccatagaaca gaagactcgg gcttttactg tgcctaatat ccccttgcaa 720
accttggtcca attctaggtt tccttccctc atccagggga tgattctgtc tcctgacgca 780
tctcaagtgg tccaattcca aaatggacgt tgcctcatag atgggtcaact cctaggcact 840
acaccgcta catcaggaca gctgttcaga gtaagaggaa agataaatca gggagcccgt 900
acgctcaacc tcacagaggt ggatggcaaa ccattcatgg catttgatc cctgcacct 960
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<210> 15
 <211> 1635
 <212> DNA
 <213> Hu/NLV/Chiba 407/1987/JP

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<400> 15
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41-19

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gtttctagat tttatcaatt aaagcctgtg ggaacagccg gaccggctag aggtaggctt 1620
ggtgtccgta gataa 1635

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<210> 16
<211> 1620
<212> DNA
<213> Hu/NLV/Narita 104/1997/JP

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ggtggagagt ttacagtatc ccctagaaac gctccgggtg agatattatg gagcgcgccc 240
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<210> 17
<211> 1647
<212> DNA
<213> Hu/NLV/Sanbu 809/1998/JP

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<400> 17
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gggaacgggc gtagaaggat tcaataa 1647

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41-20

<210> 18
 <211> 1623
 <212> DNA
 <213> Hu/NLV/Ichikawa 754/1998/JF

<400> 18
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 aatgggtgaat ttacagtttc ccctagaaat tcccctggag agatattggt caatttggag 240
 ttgggtccag aactgaaccc ttatctggca catttagcta ggatgtacaa tggttatgcg 300
 ggtggtatgg aggtgcaagt gatgctcgcg gggaacgcgt tcaactgctgg caagatcatc 360
 tttgccgcgg tggcacctta ctttccagtg gaaaatctta gcccttccca aataacaatg 420
 tttccacatg tgatcatcga tgtcagaacc ttggaacctg tattactccc aatgcctgat 480
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 atgctttaca ccccccttcg ttctaattgt tctggtgacg acgttttcac cgtctcatgt 600
 aggatcctca ctaggccctc ccctgaattt gattttacat atttgggtgcc accaacagta 660
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 ccacagaatg gtaggggtcac actagatggg gagctgttag gcacaaccca actgcaagca 840
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 taa 1623

<210> 19
 <211> 1608
 <212> DNA
 <213> Hu/NLV/Chitta 1876/1996/JF

<400> 19
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 ctaccggcc aaaacaatat tatagacccc tggattagat taaattttgt gcaggctccc 180
 aatggagagt tcacggtttc accccgcaac tcacccgggg aagtcctatt aaatttggaa 240
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 ccgattgtgg taccagctaa tggttacttc aggtttgata cttgggtcaa tcaattctat 1560
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<210> 20
 <211> 1629
 <212> DNA
 <213> Hu/NLV/Kashiwa 47/1997/JF

41-21

<400> 20

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gtggcgggac aaaccaacat aattgacccc tggataagaa caaattttgt acaagcccc 180
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ttaggaccag atctgaatcc ttatttggcc catctttcaa gaatgtacaa tggttatgct 300
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gttaggaatg tgttctatca ttttaataat caacctcaac ctagaatgag gttagtggct 540
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attcagtaa 1629

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<210> 21

<211> 1653

<212> DNA

<213> Hu/NLV/Mie 7k/1994/JP

<400> 21

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<210> 22

<211> 1626

<212> DNA

<213> Hu/NLV/Osaka 10-25/1999/JP

<400> 22

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gtcgtcgggc aacaaaatat aattgatccc tggataagaa ataattttgt gcaggctcct 180

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41-22

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gcagggtgagt ttactgtttc cccataggaat tccccctggag aaatttttgc tgaatttgga 240
ttgggaccag atttgaatcc ctacctagcc catctggccc gcatgtataa tgggcacgct 300
ggcggcatgg aagtgcaaat tgtgctggct gggaatgcgt tcacagcagg caaaatcata 360
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41-23

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Claims

1. A small round structure virus detection kit consisting essentially of :
 - a. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 1 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 1; and
 - b. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 2 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 2; and
 - c. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 3 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 3; and
 - d. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 4 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 4; and
 - e. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 5 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 5; and
 - f. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 6 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 6; and
 - g. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 7 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 7; and
 - h. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 8 or an antibody against a partial peptide

thereof having antigenicity equivalent to the peptide of SEQ ID NO: 8;
and

- i. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 9 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 9;
and
- j. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 10 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 10; and
- k. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 11 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 11.

- 2. A small round structure virus detection kit according to claim 1, wherein said antibodies have been prepared by immunizing with virus-like particles.
- 3. A small round structure virus detection kit according to claim 1, for distinguishing serotypes of small round structure viruses.
- 4. A small round structure virus detection kit for distinguishing serotypes of small round structure viruses, the kits consisting essentially of:
 - a. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 1 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 1;
and
 - b. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 2 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 2;
and
 - c. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 3 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 3;
and

- d. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 4 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 4.
5. A small round structure virus detection kit for distinguishing serotypes of small round structure viruses, the kits consisting essentially of:
- a. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 5 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 5; and
 - b. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 6 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 6; and
 - c. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 7 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 7; and
 - d. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 8 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 8; and
 - e. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 9 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 9; and
 - f. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 10 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 10; and
 - g. an antibody against a peptide having an amino acid sequence represented by SEQ ID NO: 11 or an antibody against a partial peptide thereof having antigenicity equivalent to the peptide of SEQ ID NO: 11.

6. A small round structure virus detection kit according to any one of claims 1 to 5, wherein the antibodies are immobilized on solid-phase antibody carriers suitable for capturing small round structure viruses.

FIG. 1

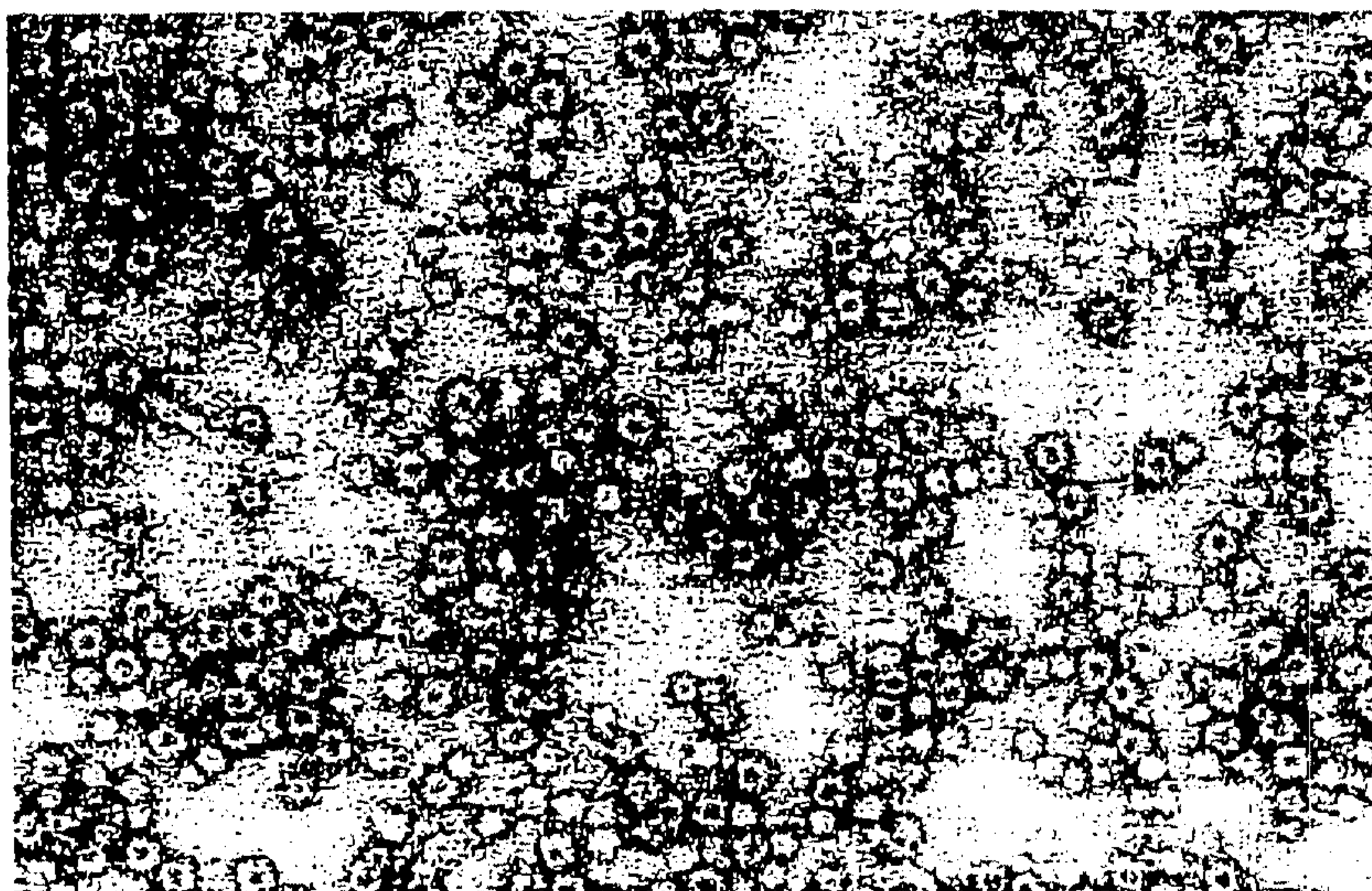


FIG. 2

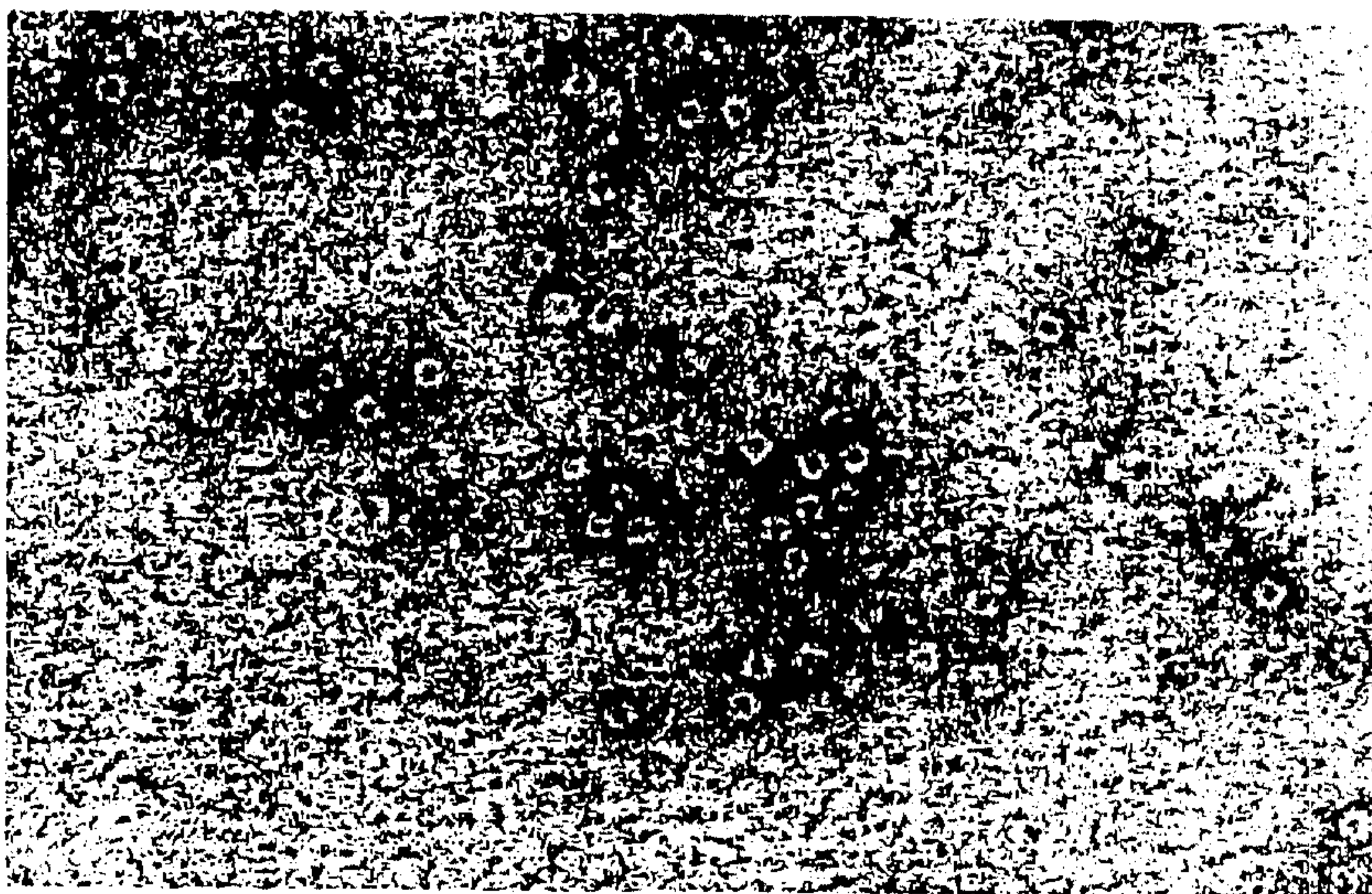


FIG. 3

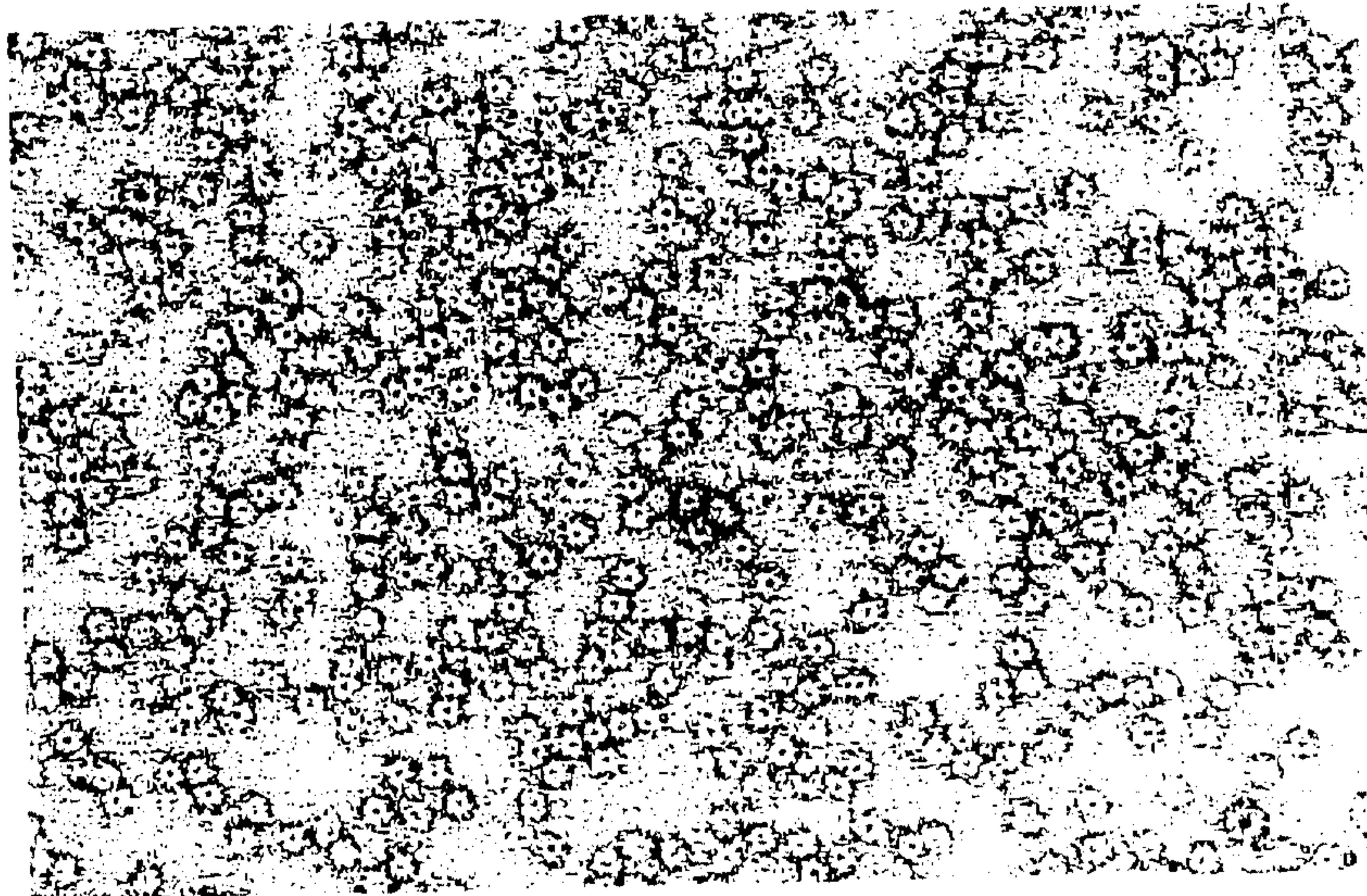


FIG. 4

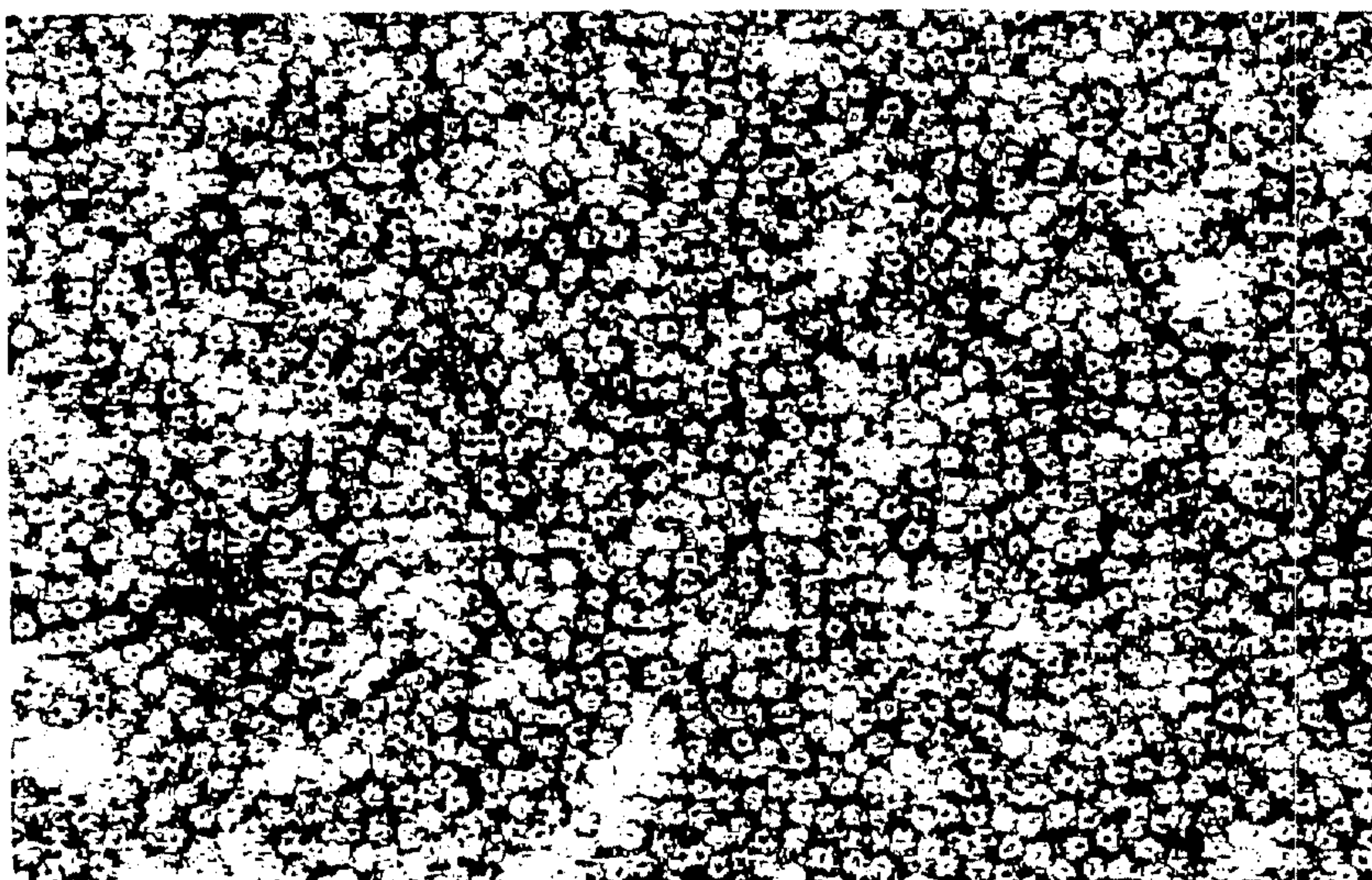


FIG. 5

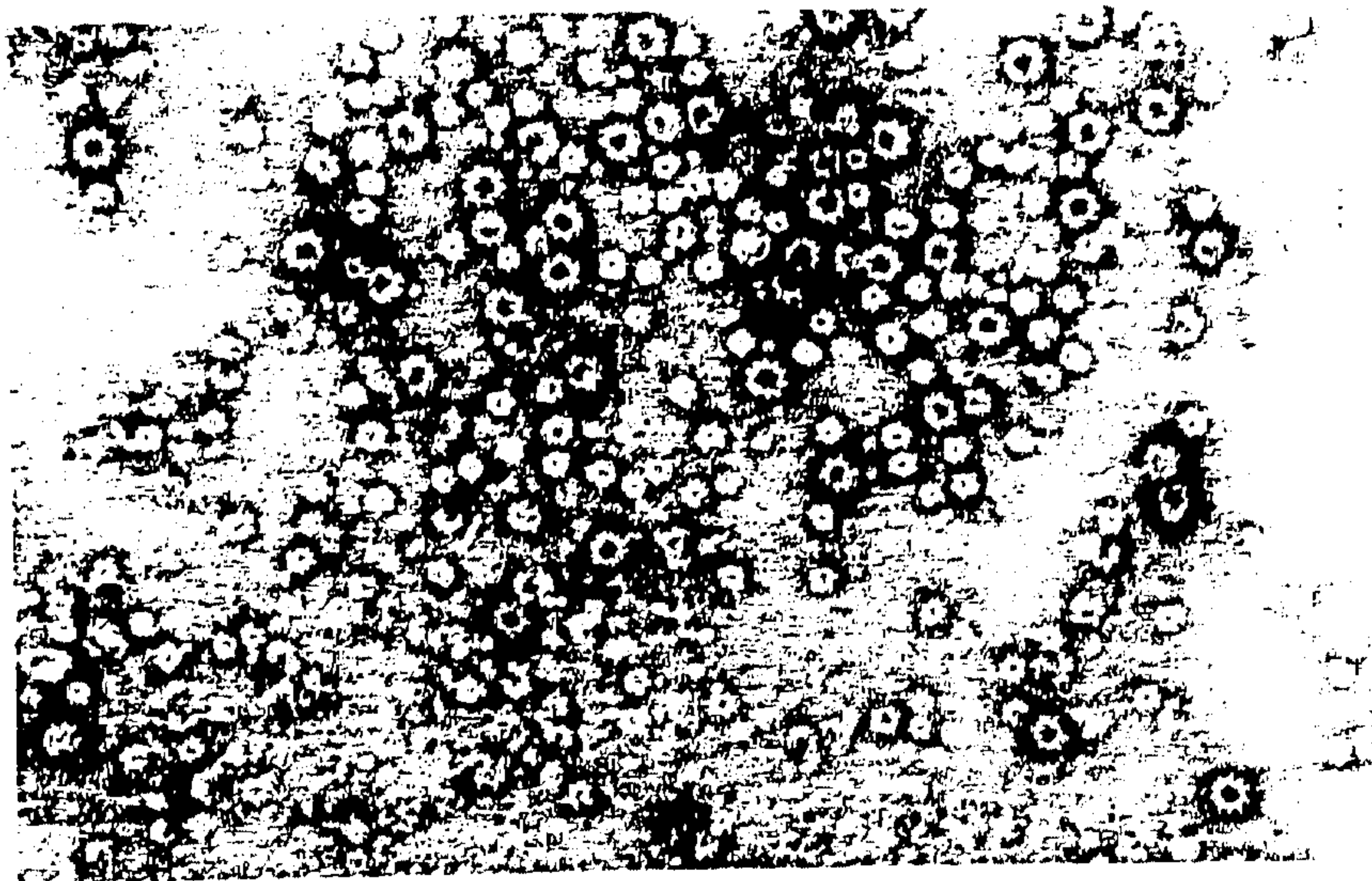


FIG. 6

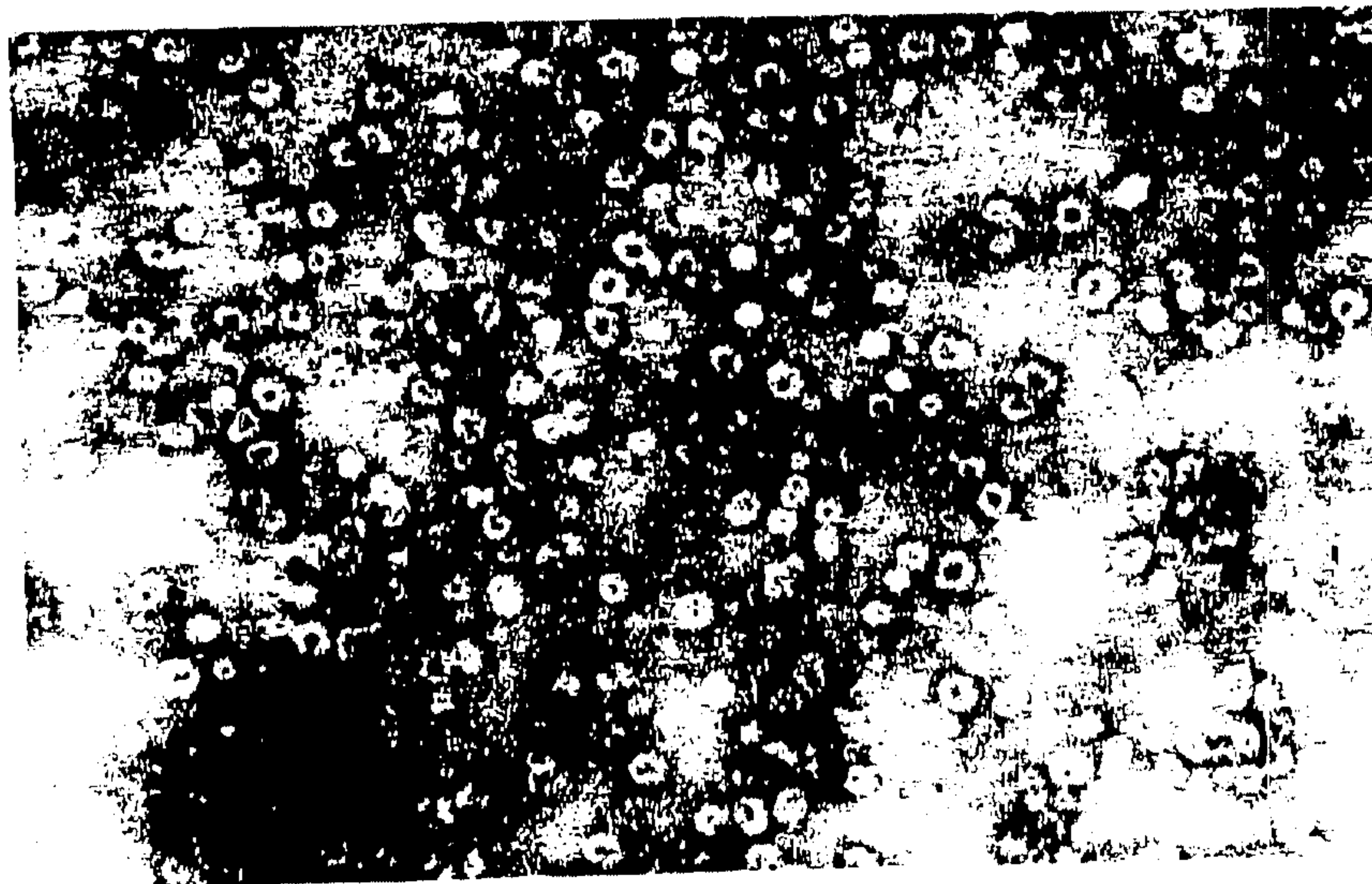


FIG. 7

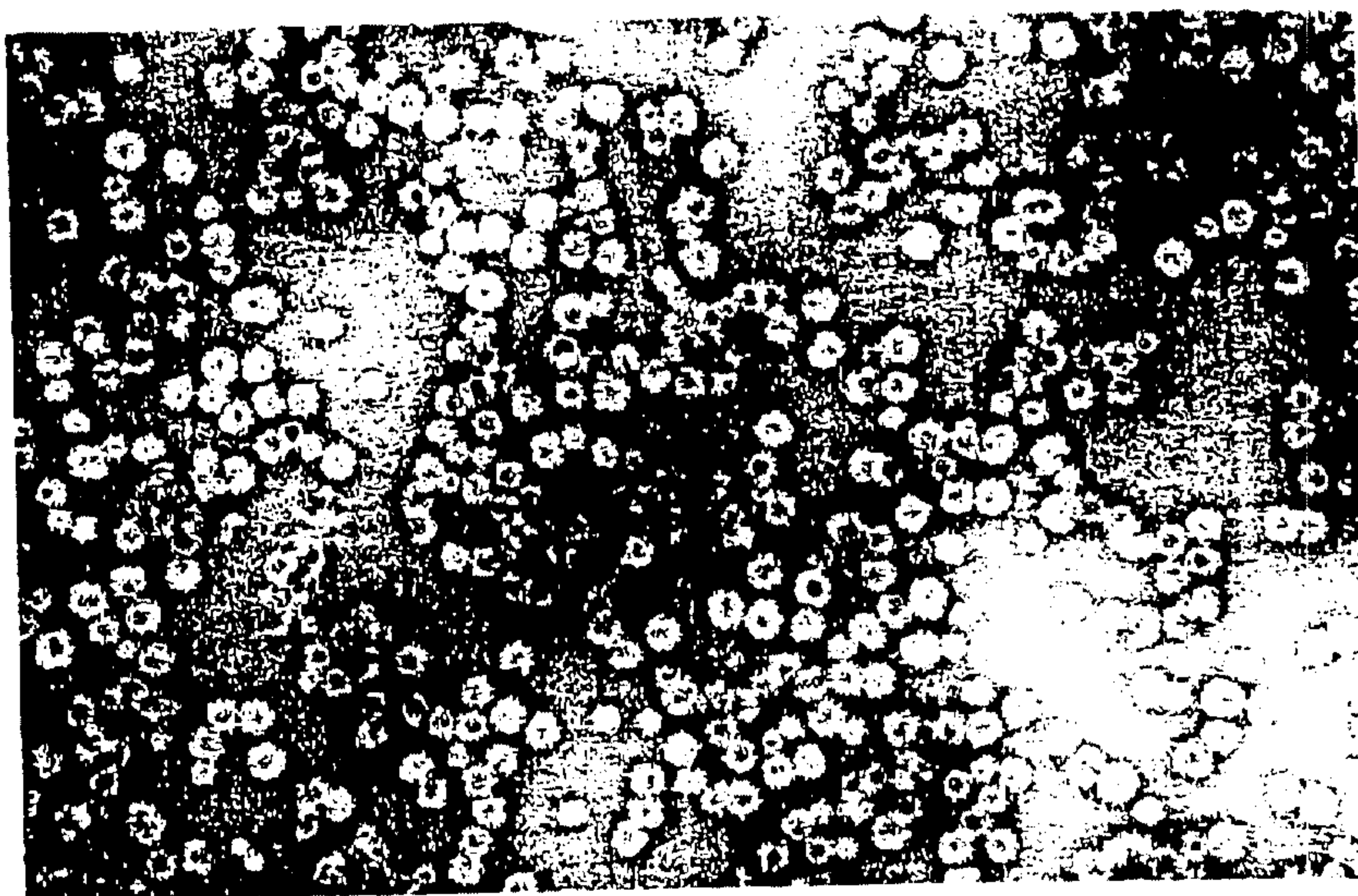


FIG. 8

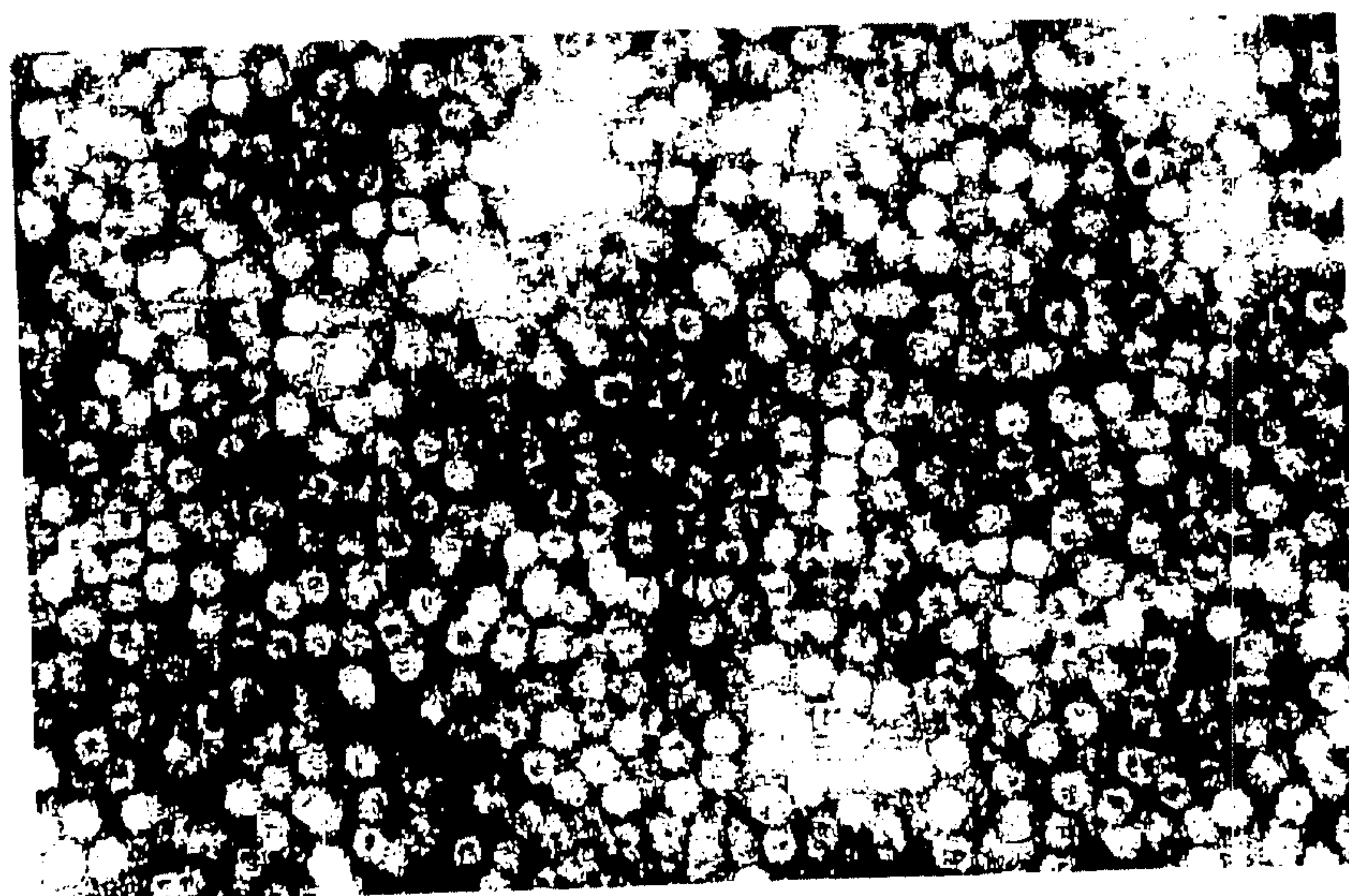


FIG. 9

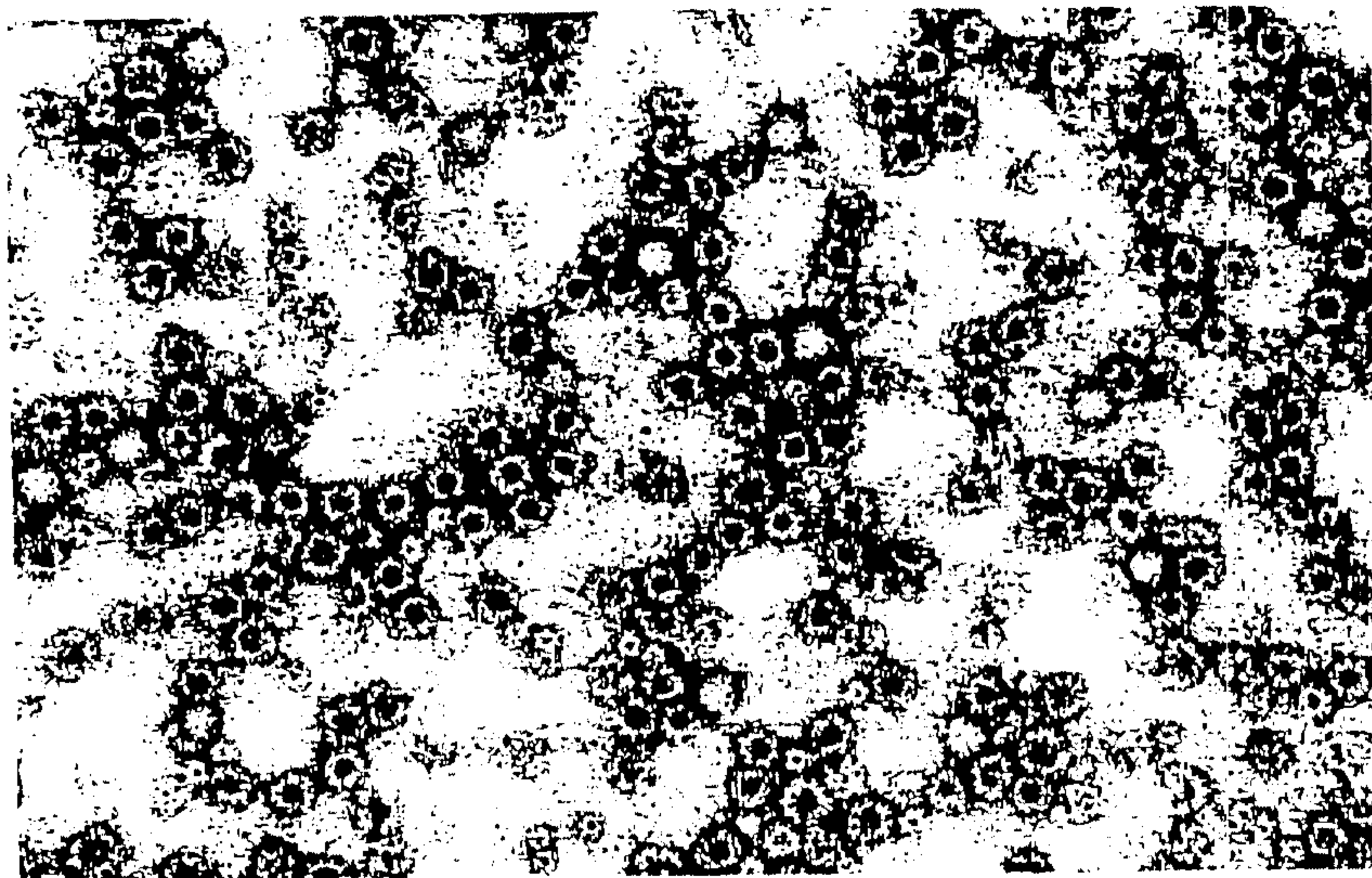


FIG. 10

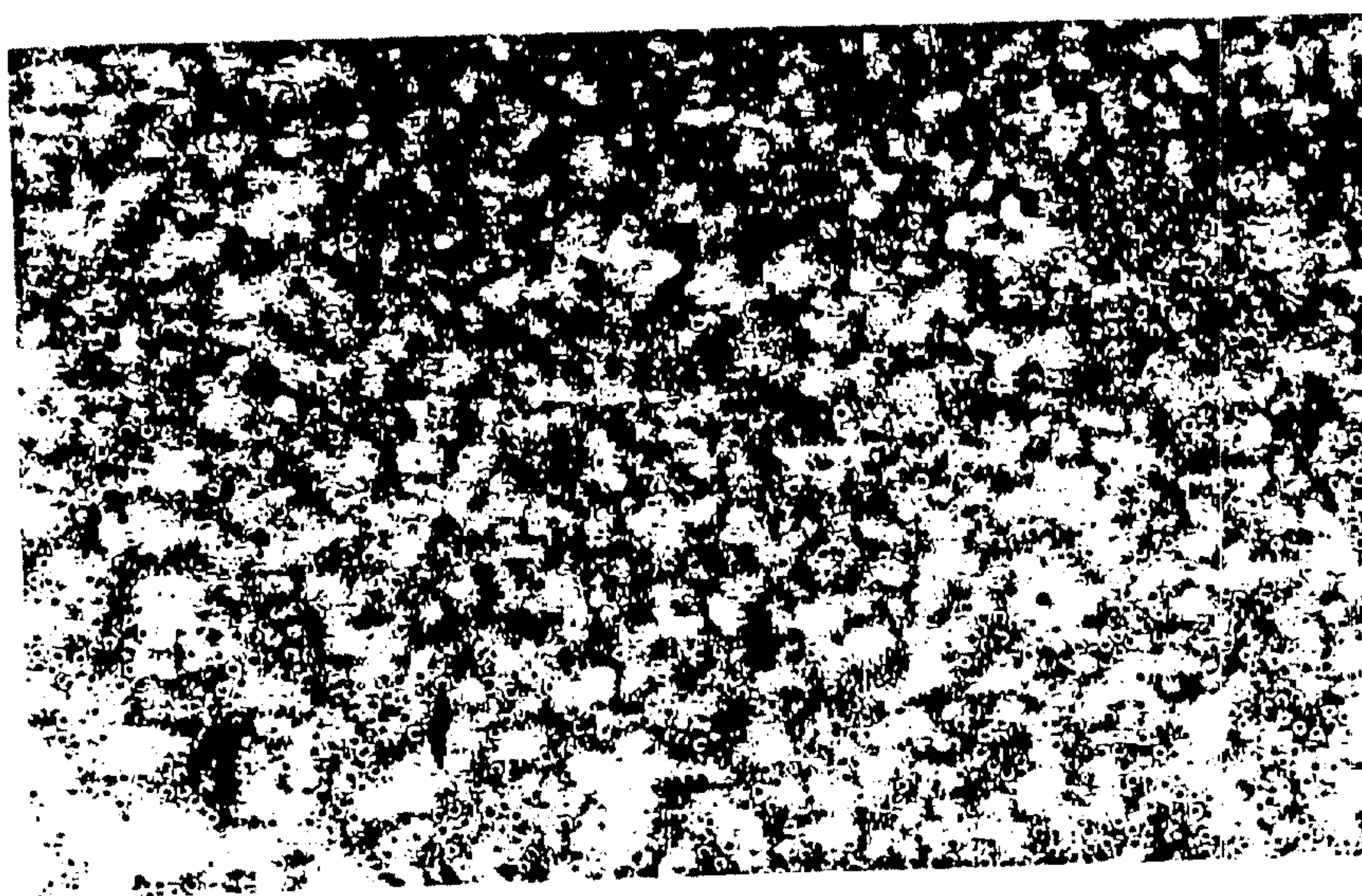
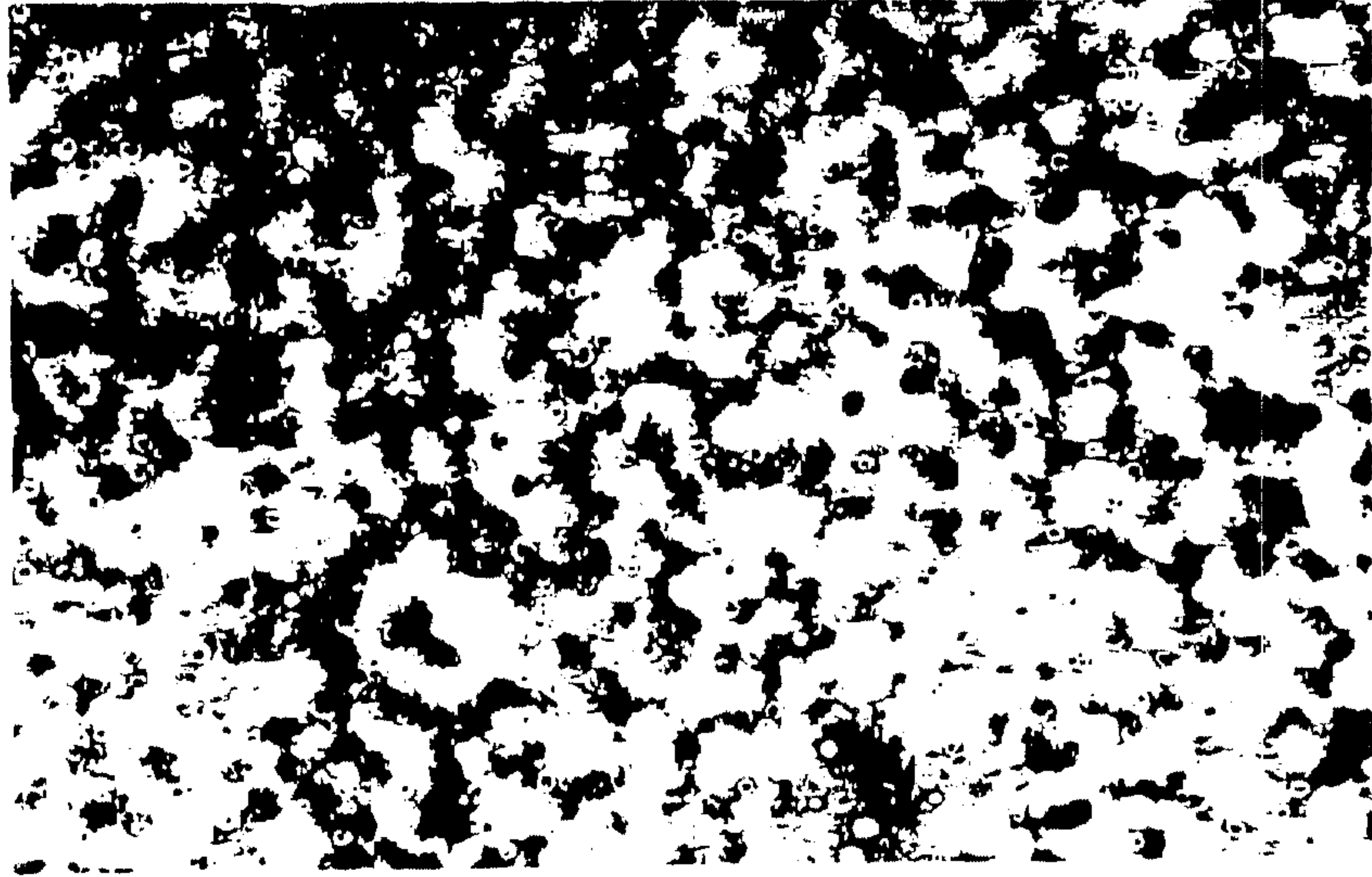


FIG. 11



Application number / numéro de demande: JP00/04095

Figures: The whole Document

Unscannable items
received with this application
(Request original documents in File Prep. Section on the 10th floor)

Documents reçu avec cette demande ne pouvant être balayés
(Commander les documents originaux dans la section de préparation des dossiers au
10^{ème} étage)