# Developing a SARS Virus Vaccine

**Dr Sergio ABRIGNANI**
Chiron Vaccines Research
Siena
**ITALY**

# SARS

An acute respiratory illness caused by infection with the **S**evere **A**cute **R**espiratory **S**yndrome **(SARS)** Corona-virus **(CoV)**

**Signs and Symptoms:** fever followed by rapidly progressive respiratory compromise

**Way of infection:** droplets, direct or indirect contacts

**Number of cases (July 2003) :** 8437 with an overall mortality of 10%

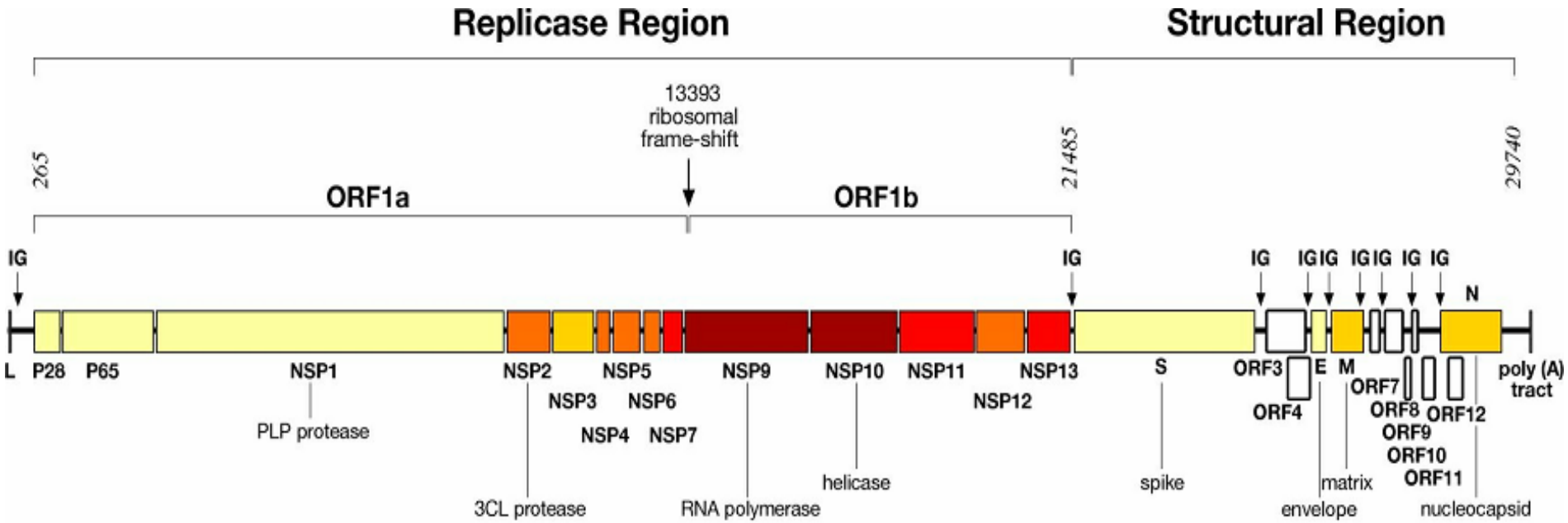
| Age | 0 - 24 | 25 – 44 | 45 – 64 | 65 - |
|---|---|---|---|---|
| % Mortality | < 1 | 6 | 15 | > 50 |

# SARS-CoV

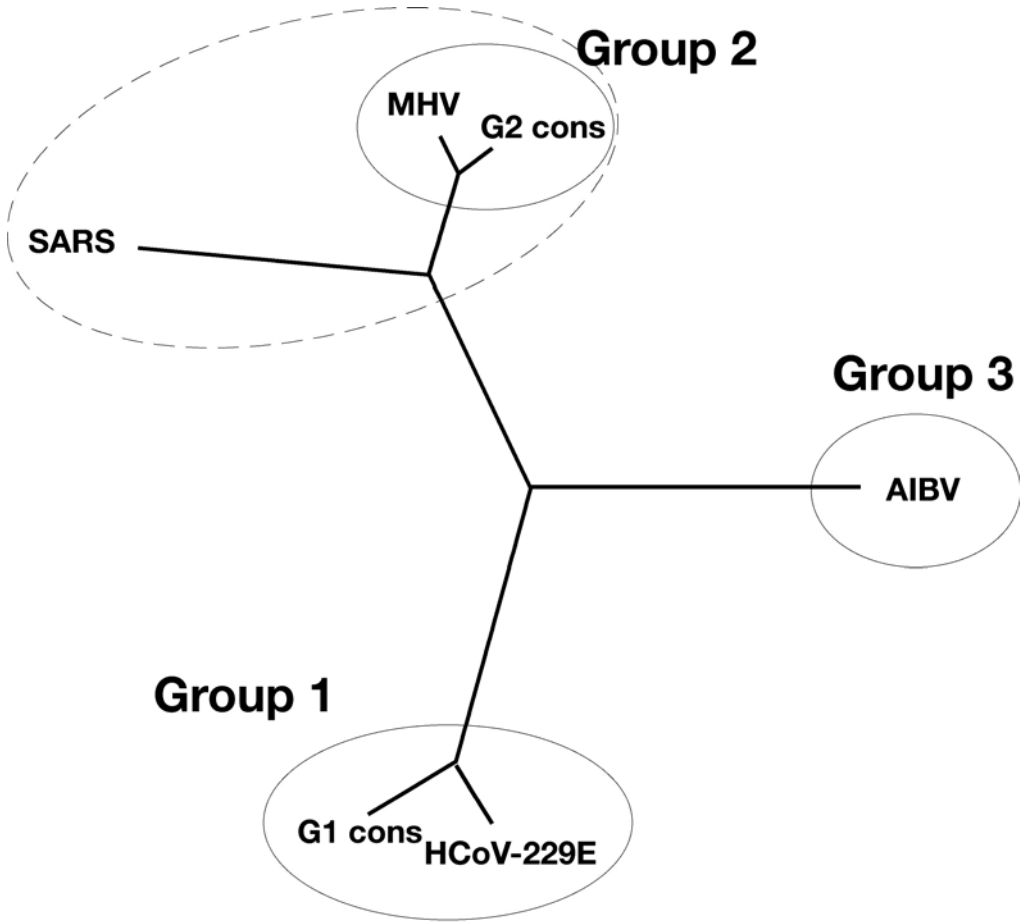
Positive stranded RNA genome of ~ 28000 bp

5 major ORFs encoding the replicase polyprotein; the spike **(S)**, envelope **(E)** and membrane **(M)** glycoproteins; and the nucleocapsid **(N)** protein

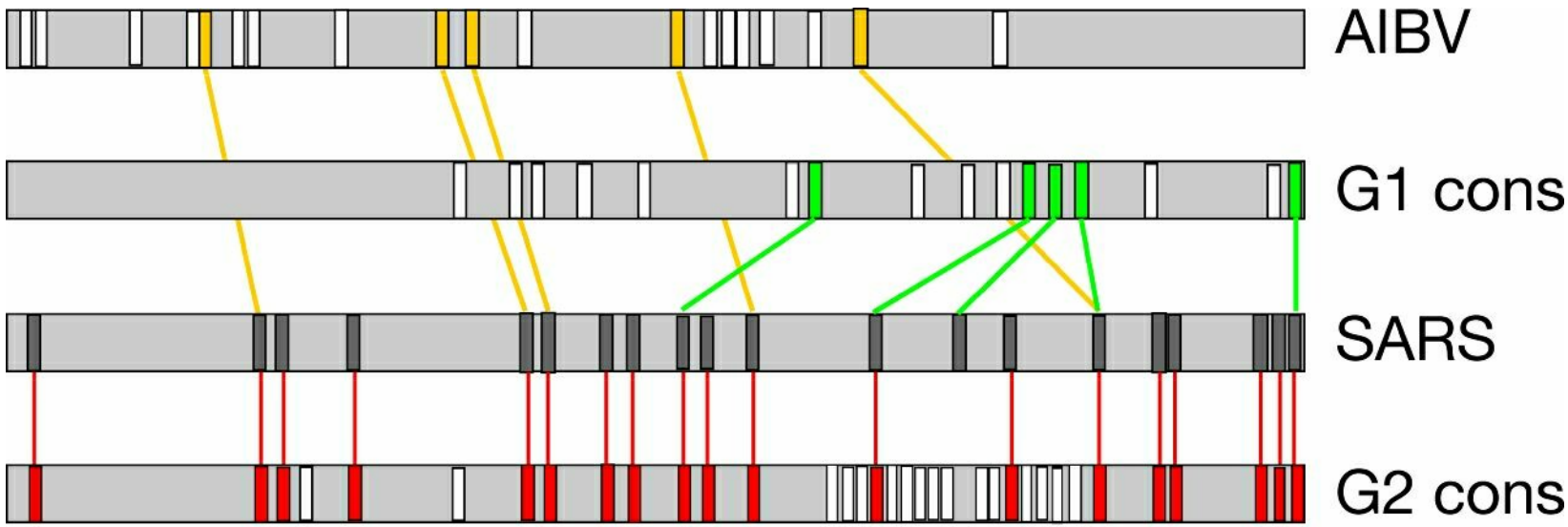
Some additional ORFs of unknown function are interspersed in the structural region



# Phylogenetic Analysis



# Cysteine Conservation in Spike S1 Subunit



# Possible Types of SARS-CoV vaccines

1. **Inactivated virus vaccine**
2. **Recombinant subunit vaccine**
   **(Spike protein)**

**Antibodies**
**CD4 T cells**

3. **Live attenuated vaccine**
4. **Vectored vaccine**
   **(Adeno-, Alpha-, Pox-virus vectors)**
5. **DNA vaccine**

**Antibodies**
**CD4 T cells**
**CD8 T cells**

# Immunity Elicited by Coronavirus Infections

1. re-infection is common (documented for human coronavirus OC43 after ~ 2 years)

2. clinical symptoms are usually reduced upon second exposure

# Examples of veterinary Coronavirus vaccines

- **avian infectious bronchitis virus (IBV):** inactivated and live attenuated vaccines show some short term protection

- **canine coronavirus (CCV):** inactivated vaccine works quite well

- **bovine coronavirus (CCV):** live vaccine shows partial protection

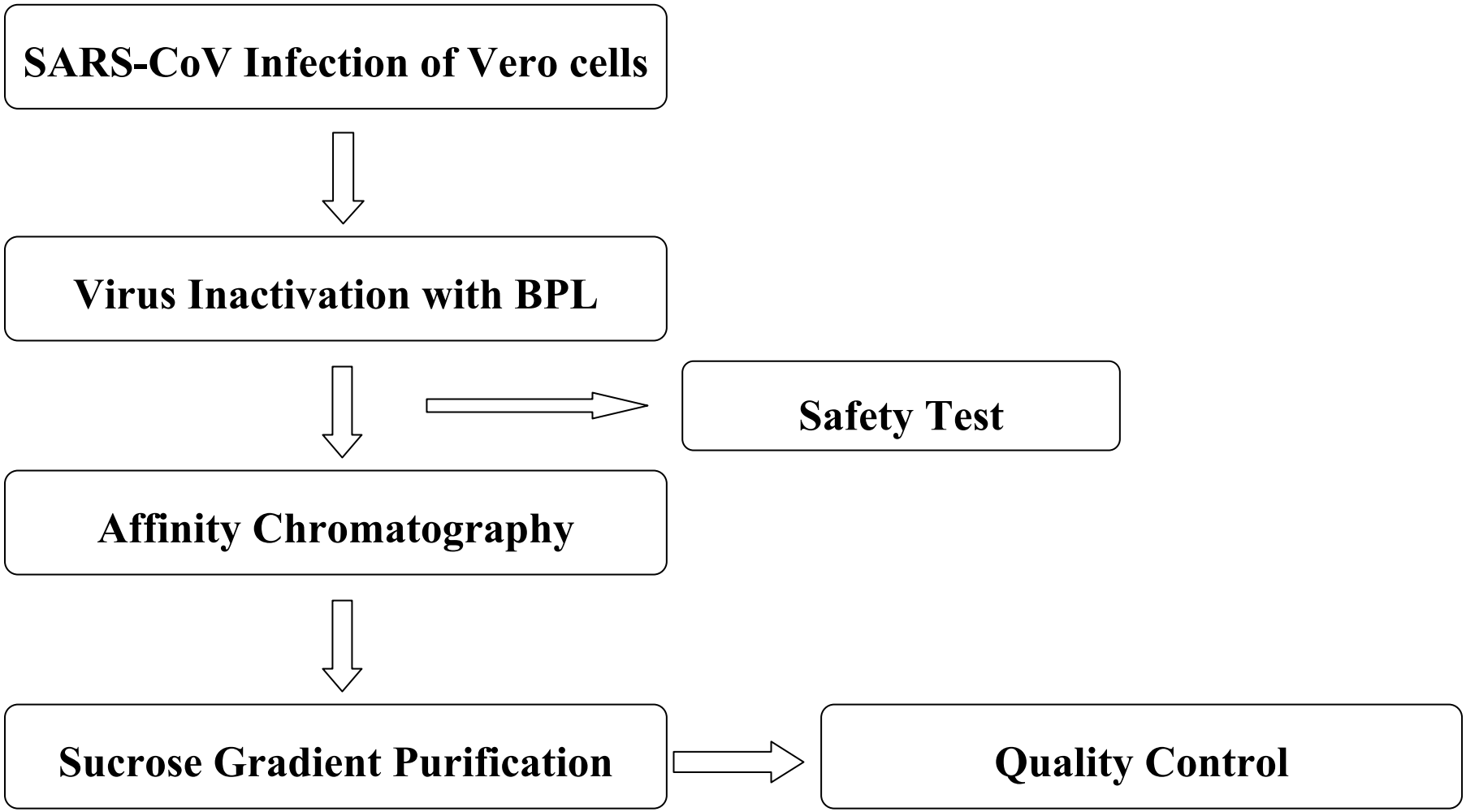
- **transmissible gastroenteritis virus (TGEV):** inactivated virus results are sub-optimal

- **feline infectious peritonitis virus (FIPV):**
  - Killed virus enhances disease
  - live-attenuated vaccines show partial protection

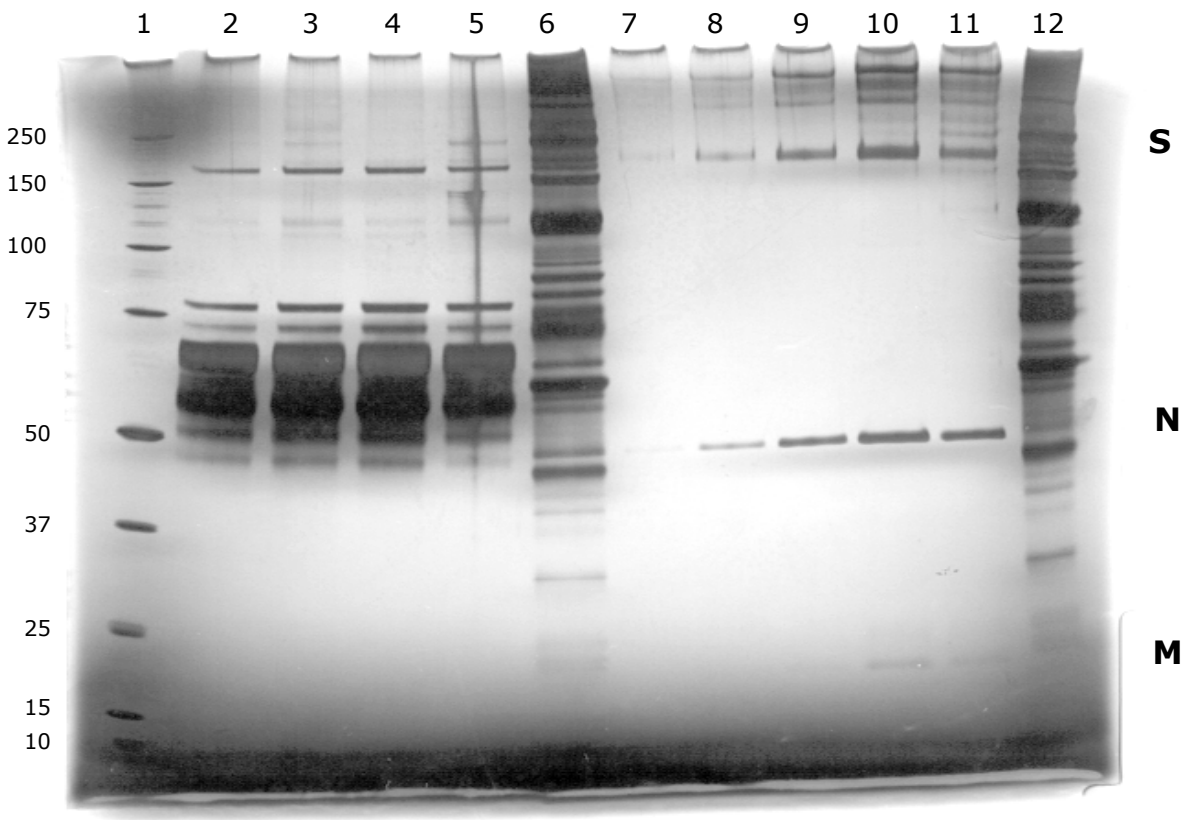
# Relevant Issues for SARS-CoV vaccine development

- Neutralizing antibodies specific for Spike protect from infection in experimental animal models
- Limited antigenic variation
- Vaccine has to protect elderly
- Vaccine should elicit mucosal immunity (also in the GI tract?)
- Vaccine should not enhance infection on exposure to wild-type virus (FIPV like)

# SARS-CoV Purification Process



# SDS-Gel Electrophoresis / Silver Stain



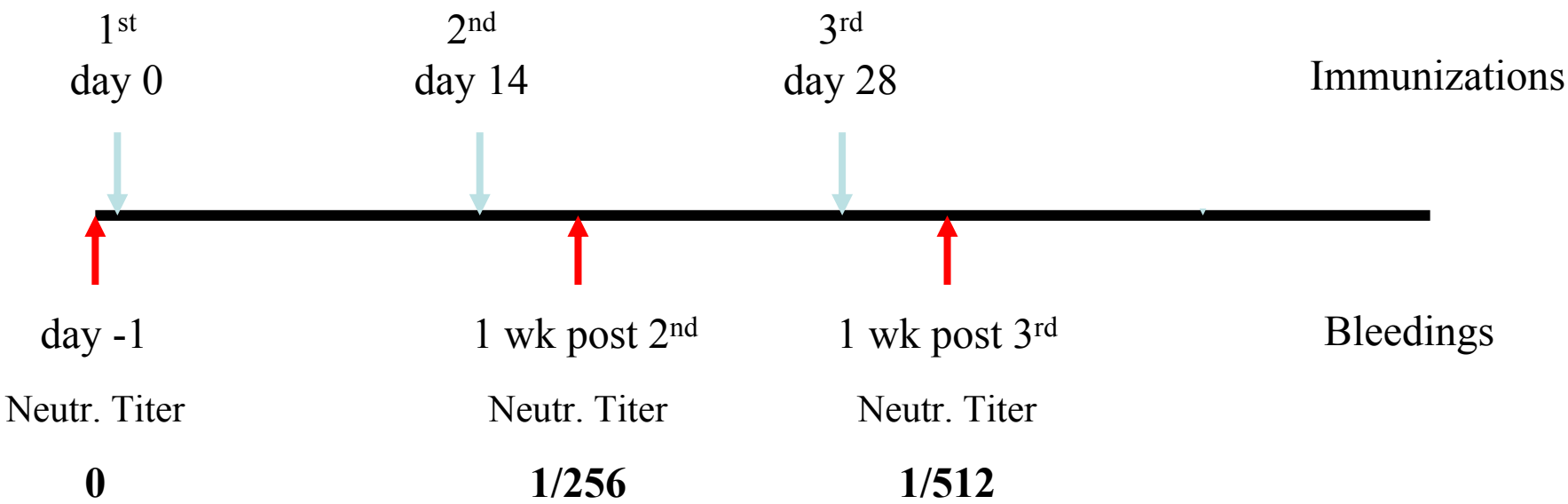

**Lane 1** Marker

**Lane 2** Harvest – Supernatant of SARS-CoV infected Vero cells

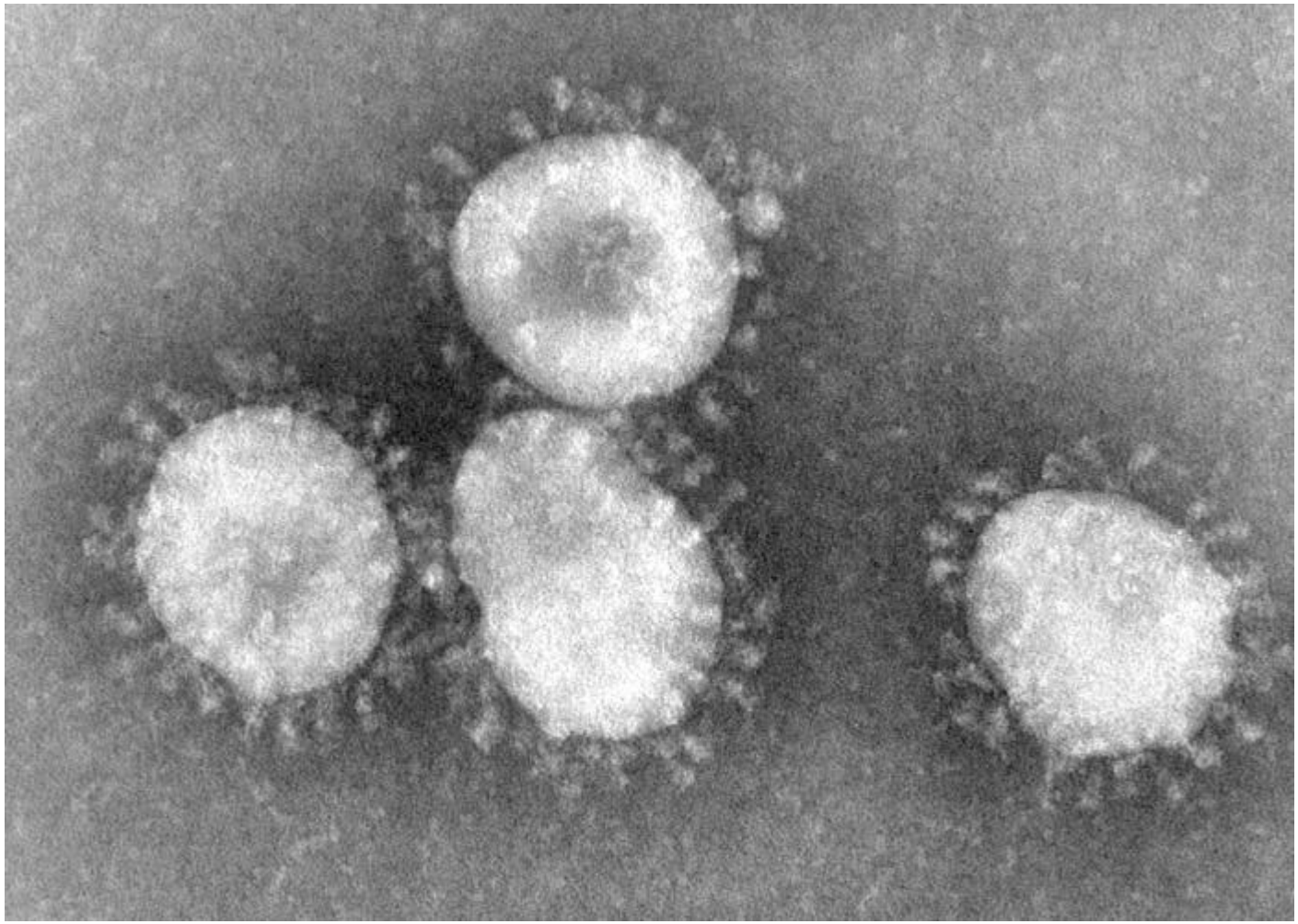
**Lane 6** Peak fraction after affinity chromatography

**Lane 9-11** Peak fraction of sucrose gradient

# Killed Virus Vaccine Elicits Neutralizing Ab

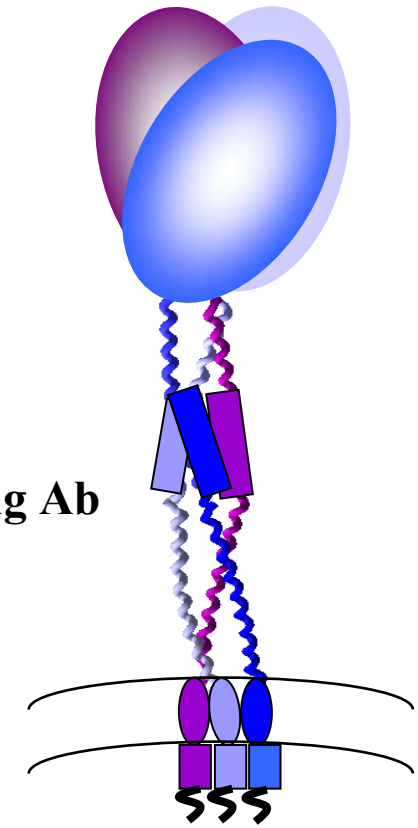



Neutralization titer in serum of convalescent patients: 1/100 – 1/1000



# Spike protein

| | |
|---|---|
| MW | ~ 180 kD |
| N-glycosylation sites | 12 |
| Quaternary structure | Homotrimer |
| Immunogenicity | Elicits neutralizing Ab |



**S1**
**receptor binding domain**

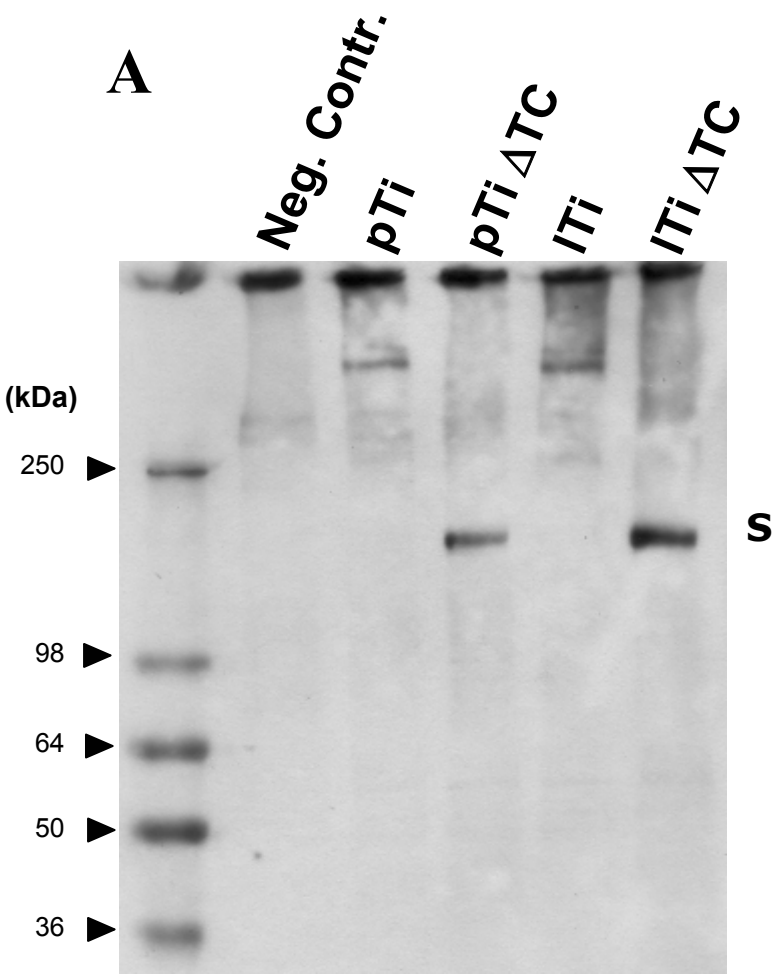
**S2**
**stem domain with fusion peptide, TM, and cytoplasmatic tail**

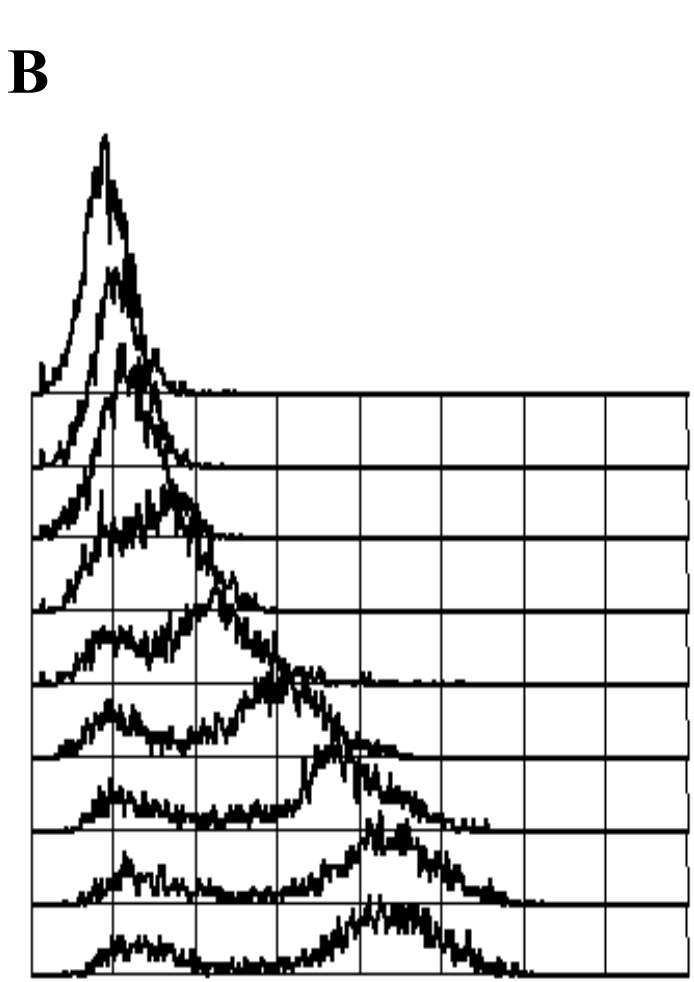
# Expression of Spike Protein in Recombinant Form

1. Mammalian cells

2. Yeast

# Western Blot and FACS Analysis of Mammalian Cells Producing Spike Protein



# SARS Vaccine Candidate Selection

- Immunogenicity in small animals
- Serum of vaccinated animals is tested for neutralizing antibodies *in vitro*
- Assess efficacy in animals (mice and/or macaques)

# SARS Vaccine Efficacy Trial

- Select high risk population
  (health care workers in the Far East during epidemic season)
- Generate incidence data in two consecutive years
- Decide clinical trial size
- Make phase III trial

# Conclusions

- An inactivated SARS-CoV vaccine induces in mice neutralization titers comparable to those elicited by natural infection in man
- We are currently testing the efficacy of an inactivated SARS-CoV vaccine in an animal model of infection
- Recombinant spike protein has been expressed in mammalian cells with the aim of developing a recombinant SARS vaccine

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