ทำอย่างไรงานวิจัย “ไม่” ขึ้นหิ้ง

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ศาสตรเมธาจารย์ ศาสตราจารย์วิจัยดีเด่น เมธีวิจัยอาวุโส

ที่ปรึกษาด้านงานวิจัย คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

MMS Mahidol Dec 17th, 2020

Research Area

- Diagnostics / Test kits
- Vaccine development
- Therapeutic antibodies
- Allergy

1. ชุดตรวจวินิจฉัยโรคติดเชื้อในเขตร้อน

Test kit development

- Parasitic infections
- Food pathogens
- Pathogens causing febrile illness
- Allergen detection/Allergen quantification

- Amoebic liver abcess
- Gnatostomiasis
- Angiostrongyliasis
- Paragonimiasis
- Trichinellosis
- etc.

Pathogens causing fever

- *Salmonellosis / typhoid*
- Scrub typhus
- Leptospirosis
- Influenza

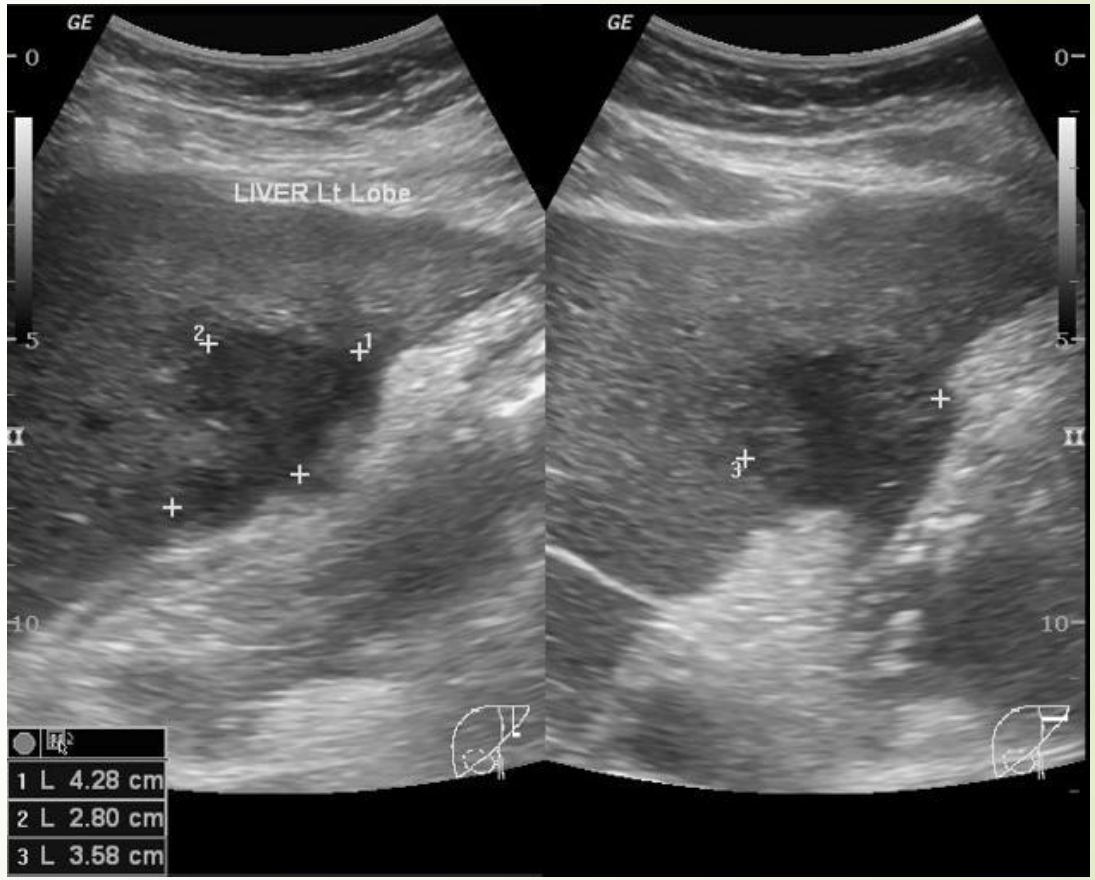
Food pathogens

- *Salmonella*
- *V. cholerae* serogroups O1 & O139
- *Listeria* spp.
- Enterotoxigenic *E. coli*
- Enterohemorrhagic *E. coli*

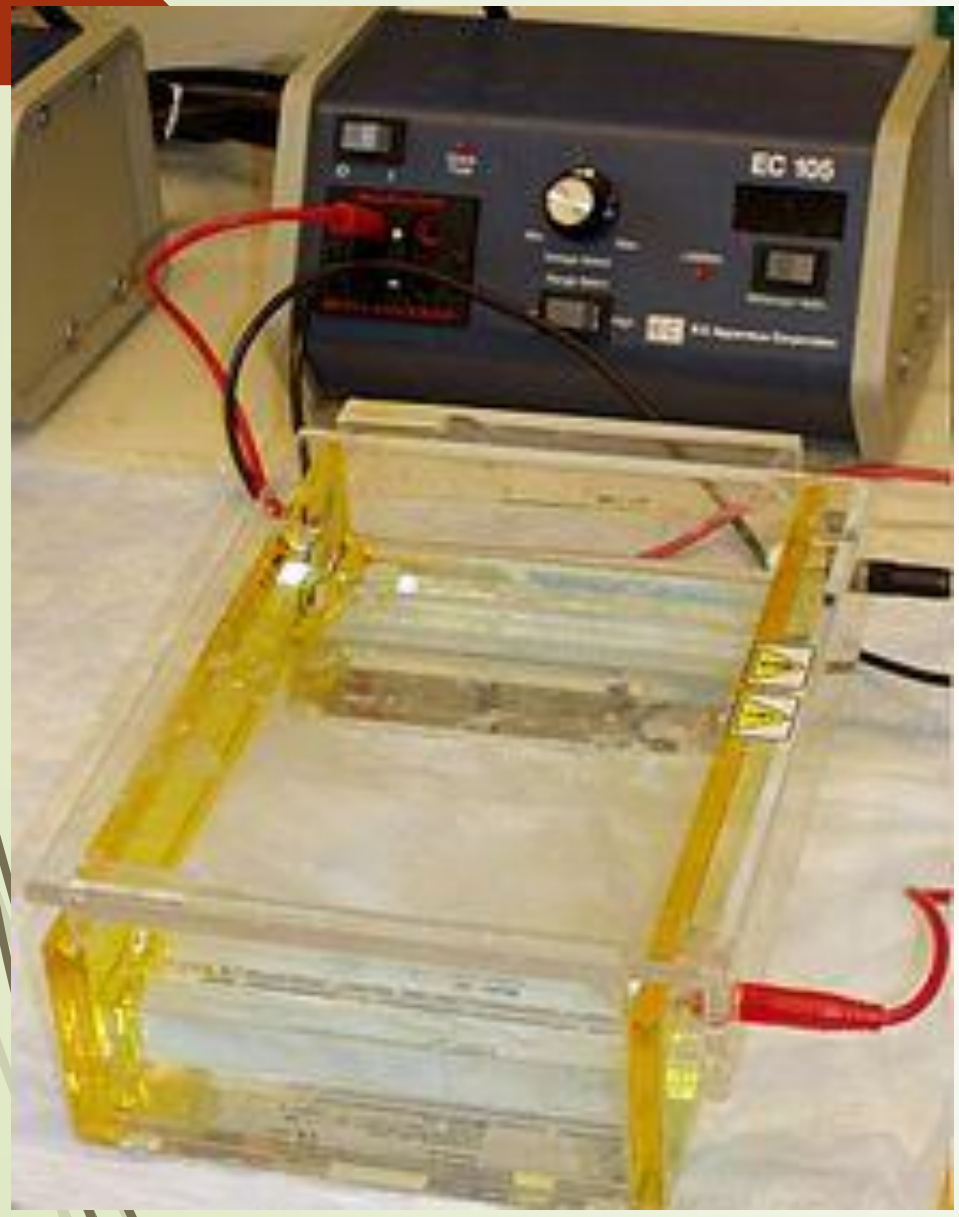
- Allergen

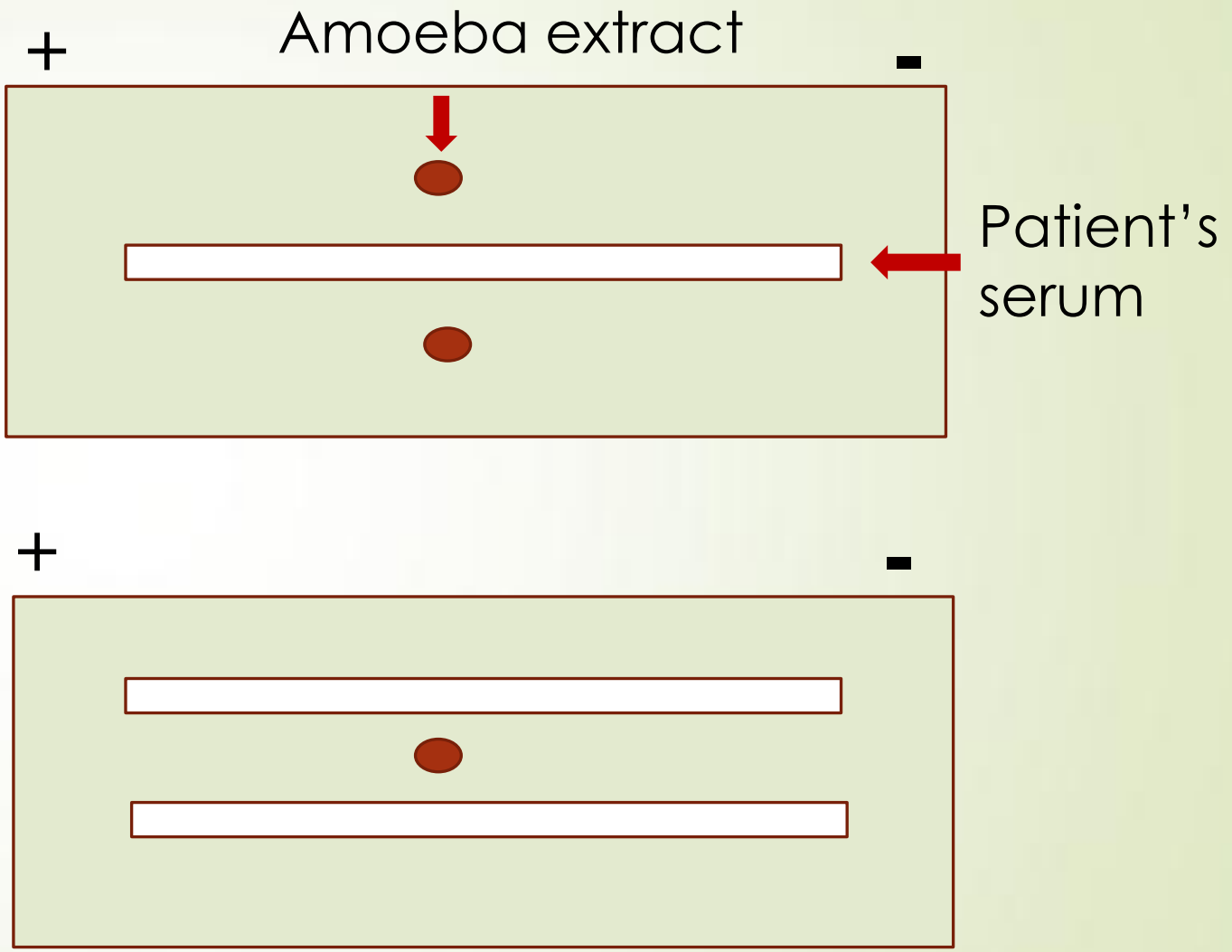
2509/1966: คณะอายุรศาสตร์เขตร้อน มหาวิทยาลัยแพทยศาสตร์

Entamoeba histolytica cyst stained with iodine

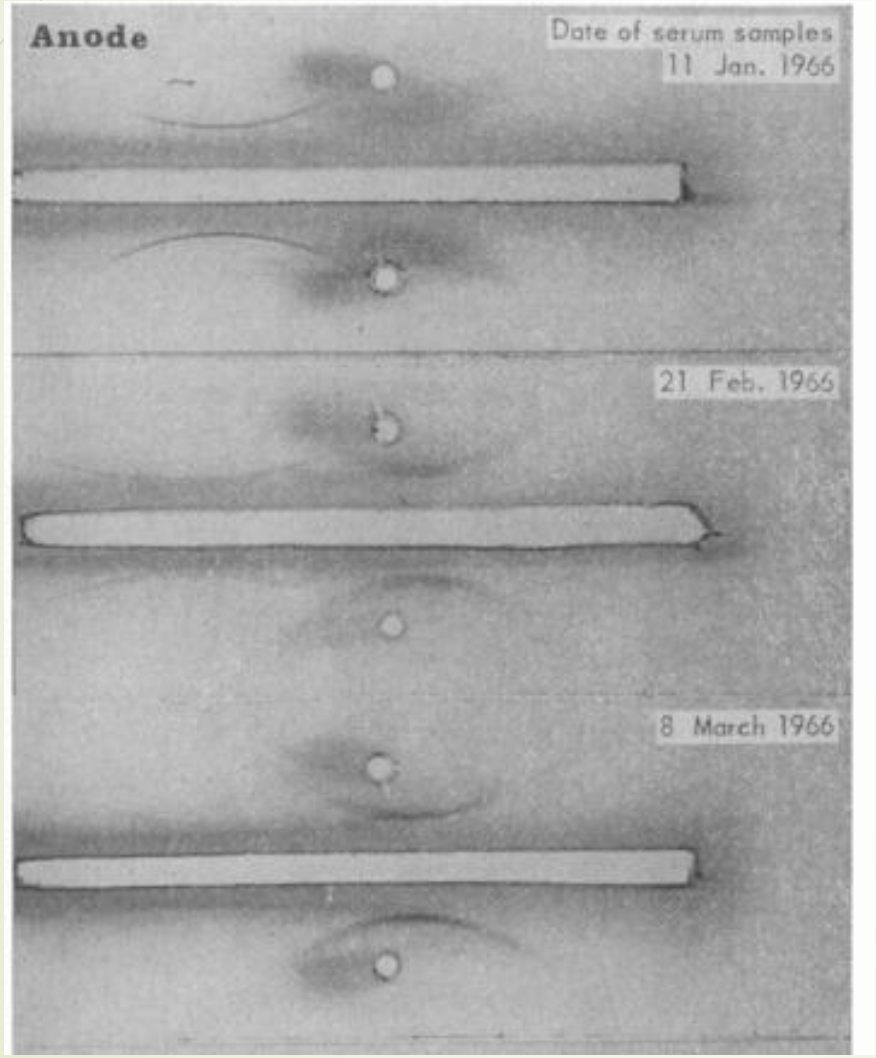


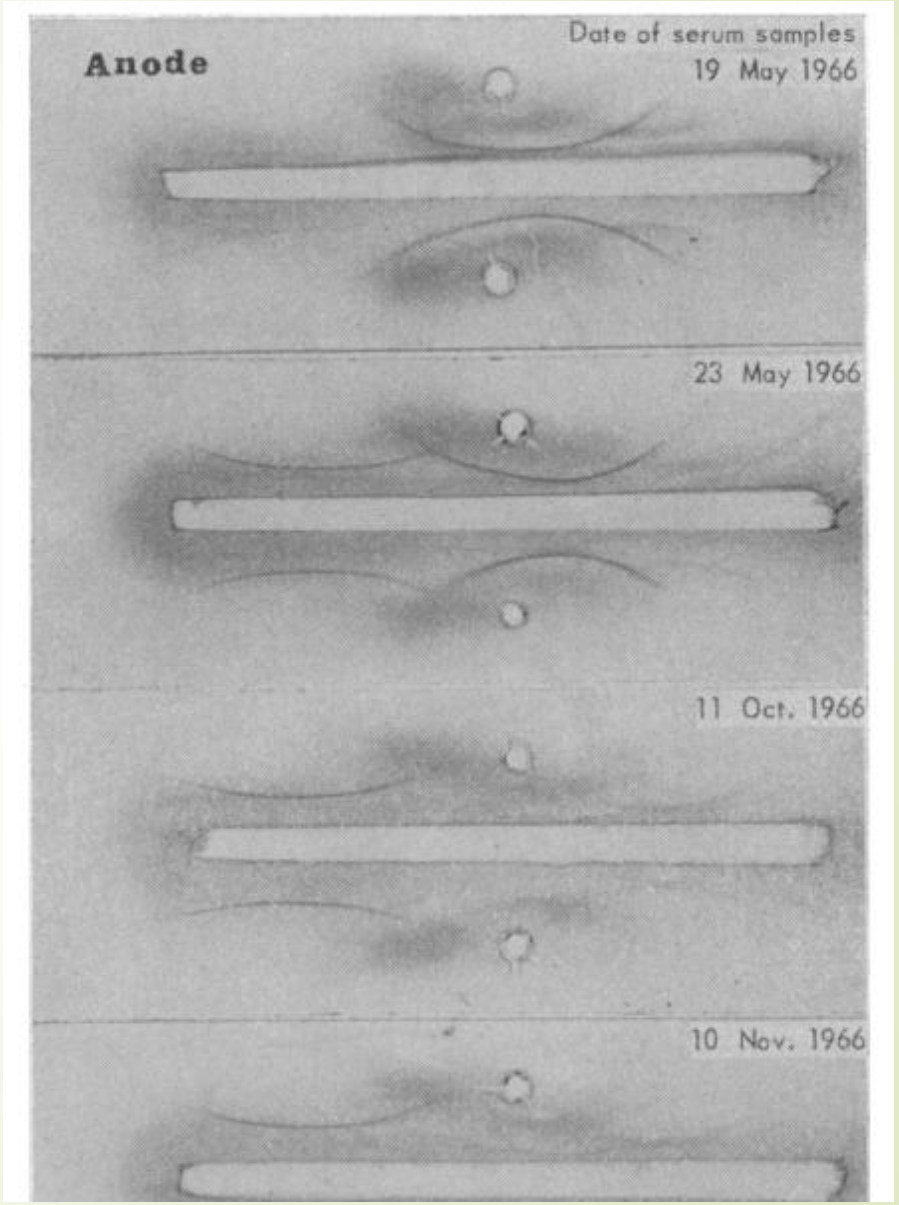
Amoebic liver abscess


EC 105


+
Amoeba extract
-
Patient's serum
+
-

Immunoelectrophoretic patterns of antibody in sera of patients with amoebic liver abscess





รางวัลมหาวิทยาลัยมหิดล สาขาการวิจัย 2518

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Immunoelectrophoresis Test for Amoebiasis*

T. SAVANAT, M.B., Ph.D. & WANPEN CHAICUMPA, D.V.M.

Haemagglutination and immunoelectrophoresis tests were investigated to find which was more suitable for the immunodiagnosis of amoebiasis. Both tests were positive in more than 90 % of sera from patients with amoebic liver abscess. With serum from blood donors and patients with other diseases a much lower percentage of positives was given by the immunoelectrophoresis test, showing that this test had a closer correlation with clinically important disease.

The immunoelectrophoretic patterns were of several varieties, but a single prominent band located near the well was considered as characteristic of amoebiasis.

Follow-up studies showed that both haemagglutinating and precipitating antibodies persisted for several months, accompanied in certain patients by changes in the immunoelectrophoretic pattern. Antibody activities were shown by means of column chromatography and "reversed" immunoelectrophoresis to be associated with serum IgG.

Although the parasitological diagnosis of amoebiasis is satisfactory in intestinal forms of the disease, it is not satisfactory in cases of liver abscess. Amoebae are often not found in the aspirated pus, and the diagnosis of amoebiasis is usually made presumptively on the finding of sterile pus of characteristic appearance. In patients with abscesses that are deep, small or multiple, the diagnosis is often delayed owing to difficulty in obtaining pus, sometimes with unhappy consequences. The need for sensitized red blood cells is required for each performance of the test. It is obviously uneconomical when only a few serum samples are tested at a time. Furthermore, the persistence of the haemagglutinating antibody long after the actual infection has subsided makes it difficult to differentiate between present and past infection.

By virtue of its better resolution, immunoelectrophoresis (IEP) is theoretically considered to be superior to the gel-diffusion test. The IEP was used

Life Cycle of *Gnathostoma spinigerum*

พยาธิตัวจี๊ด *Gnathostoma spinigerum*

Definitive host
(cat, dog, tiger, leopard)

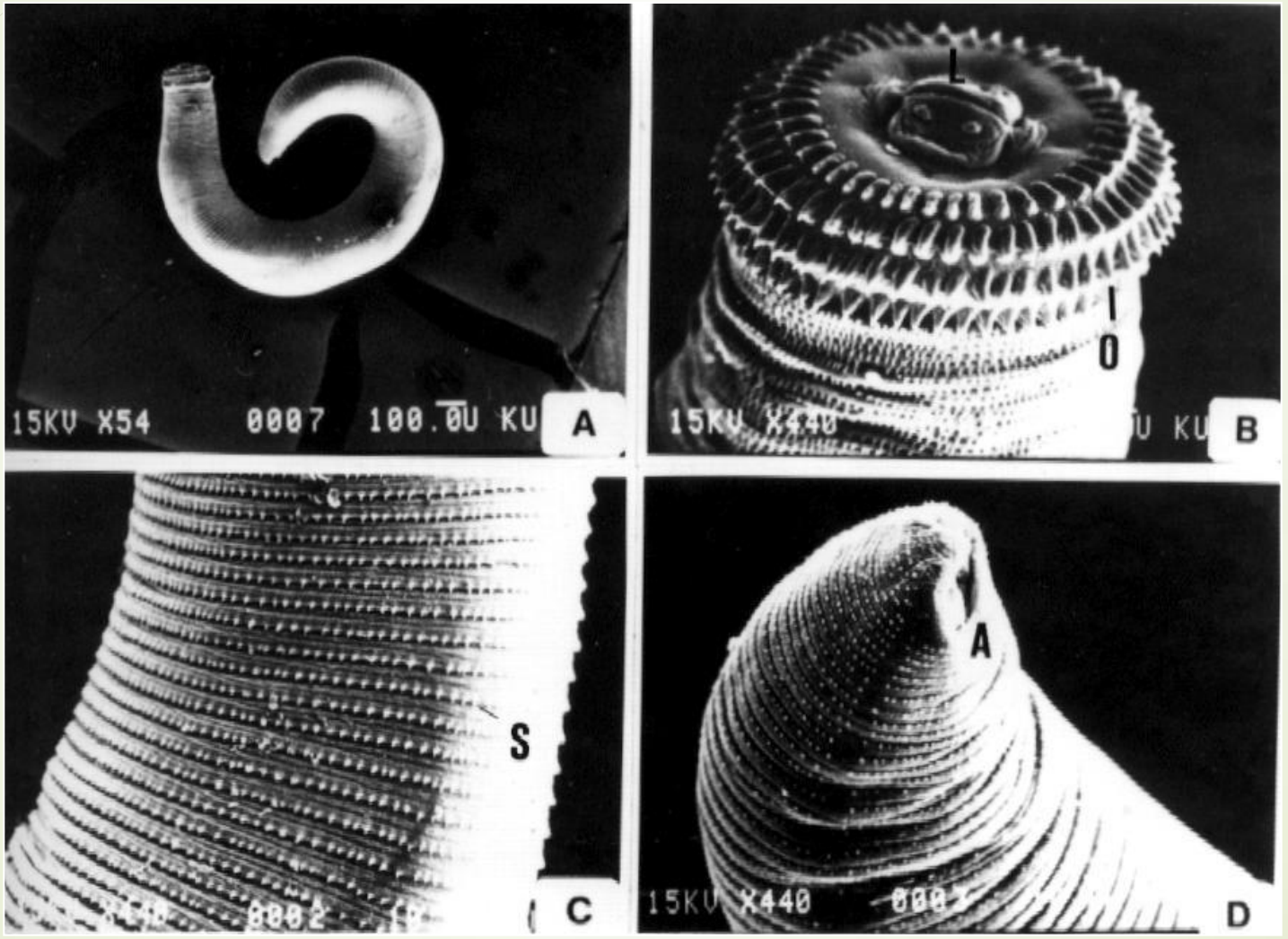
eggs and
1st stage larvae
in **fresh water**

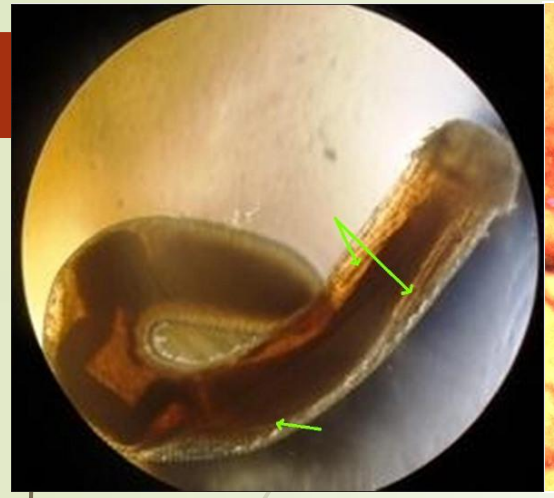
2nd stage & early 3rd stage larvae
in **cyclops**

Accidental host (man)

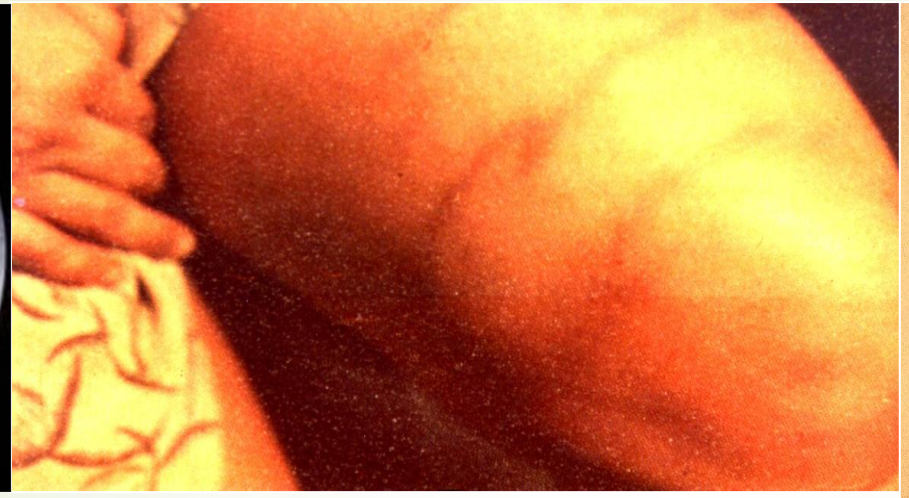
advanced 3rd stage larvae
in **fresh-water fish, amphibian,**
reptile, avian, mammal

Gnathostomiasis: *Gnathostoma spinigerum*





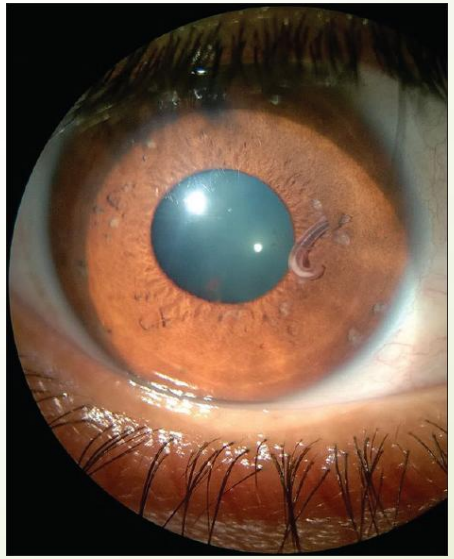
Occular gnathostomiasis



Cutaneous migratory swelling

1. Brain hemorrhage, meningitis, encephalitis
2. Occular gnathostomiasis: Blindness
3. Others

Cutaneous gnathostomiasis



opthalmitis

Occular gnathostomiasis

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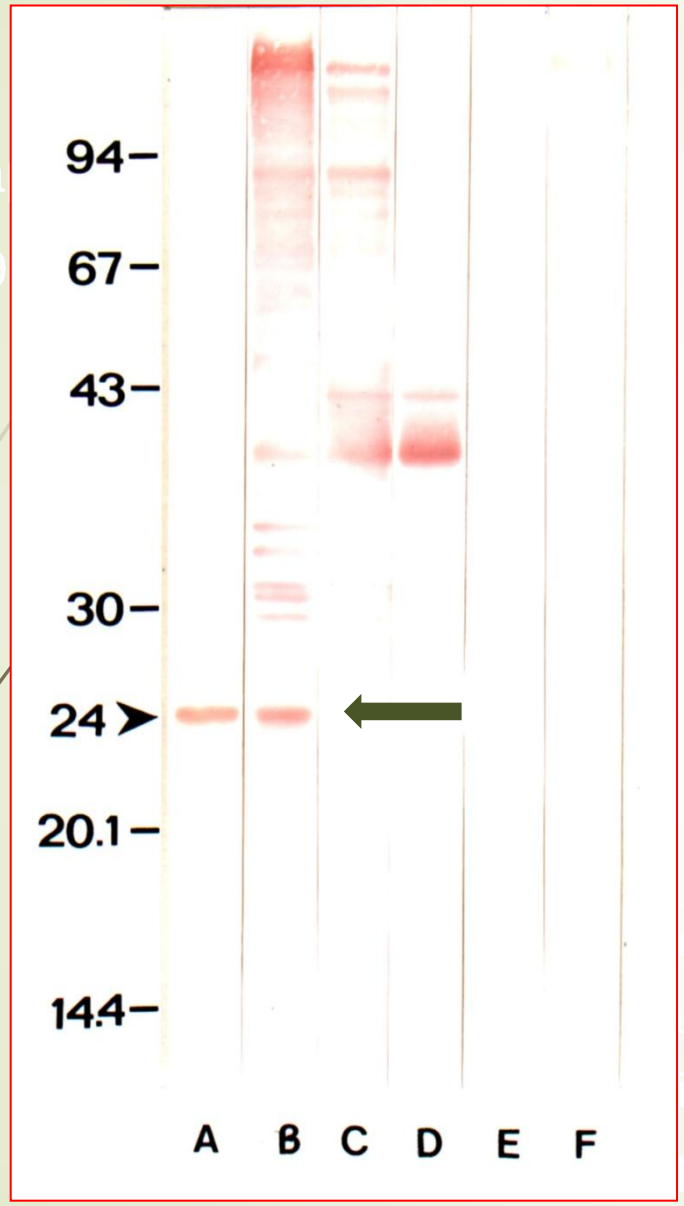
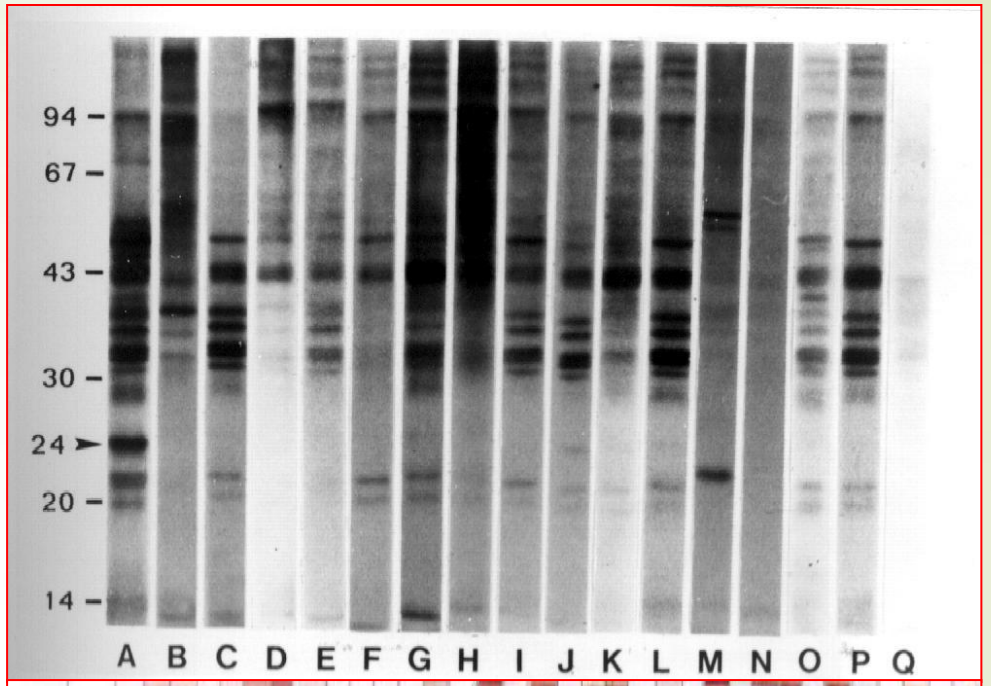
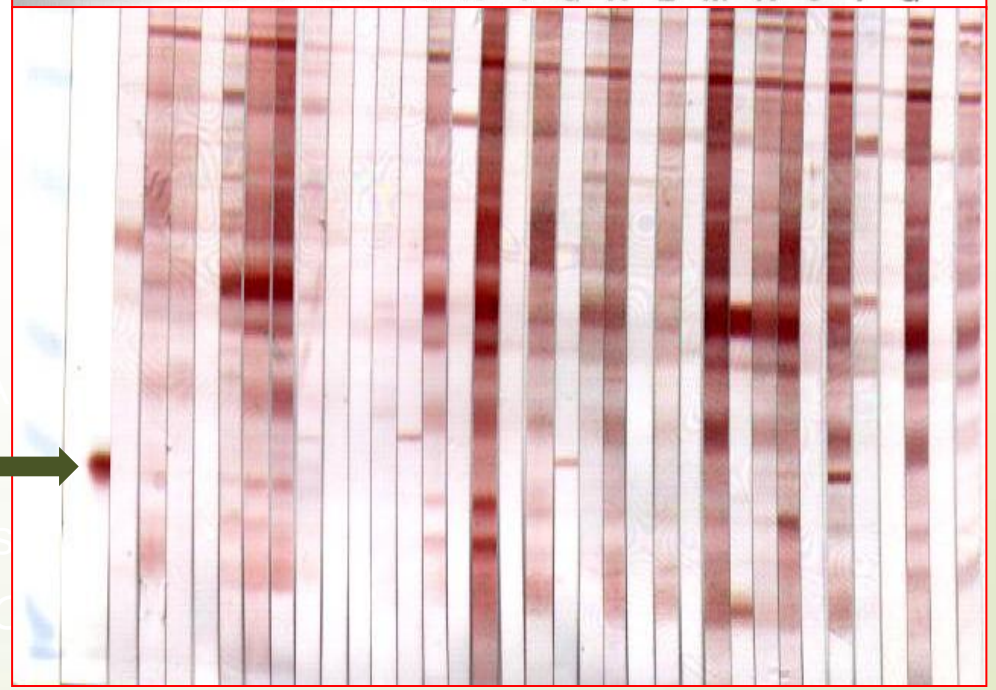
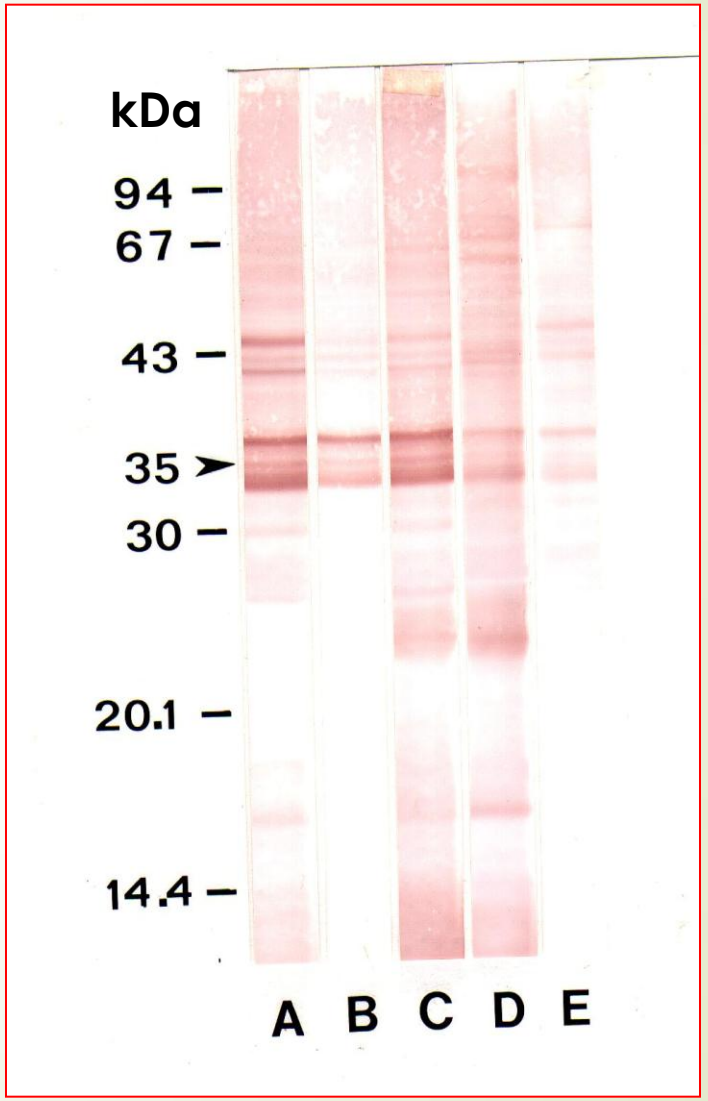




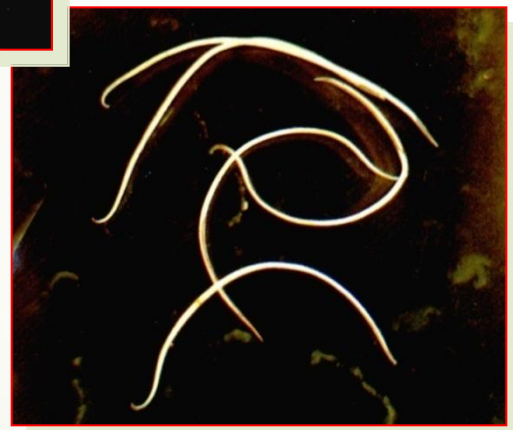


พยาธิใบไม้ปอด Paragonimiasis



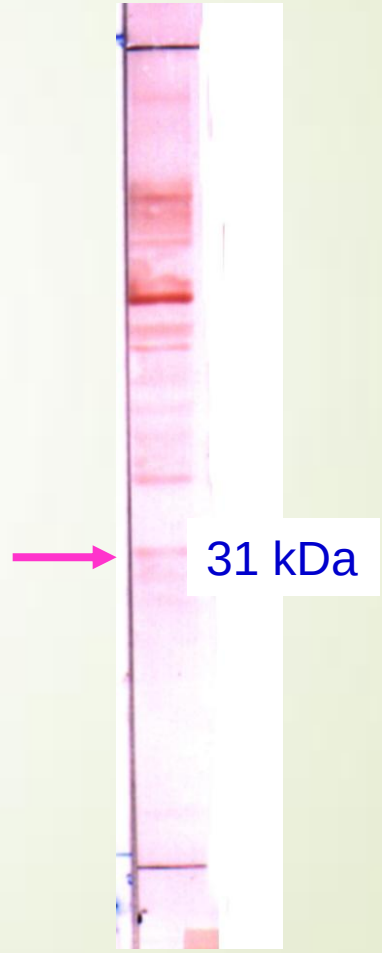
พยาธิหอยโข่ง (*Angiosstrongylus cantonensis*)

female



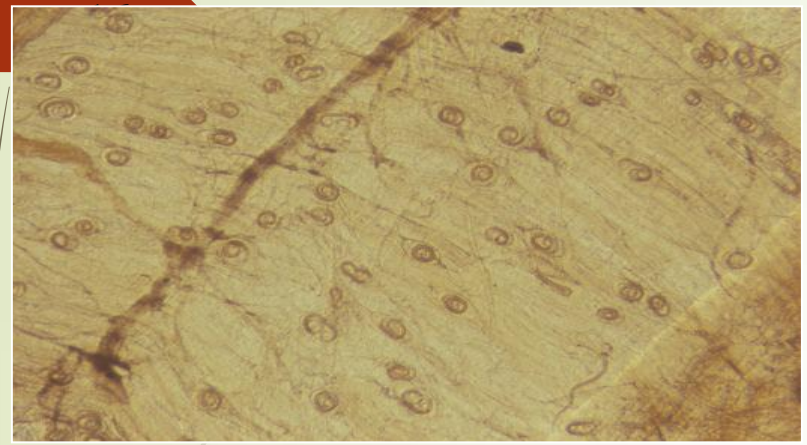
male

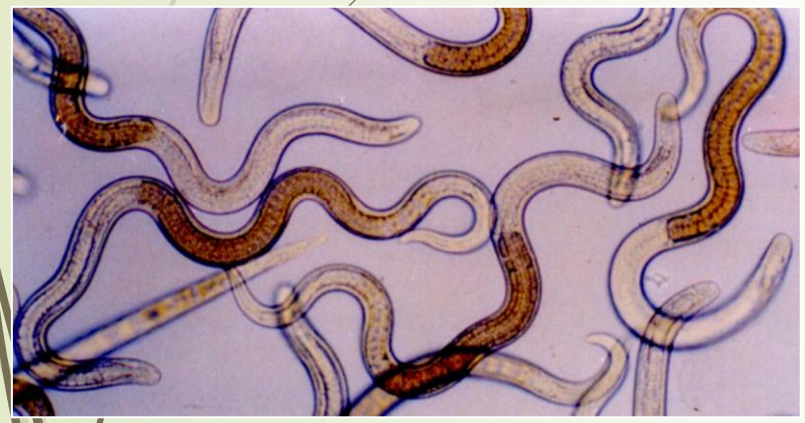
Infective stage larva

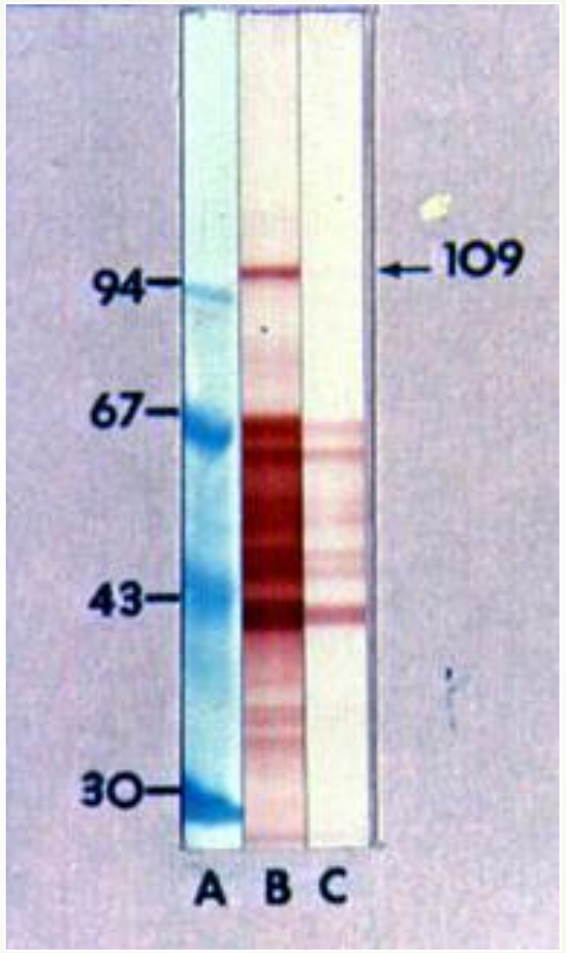


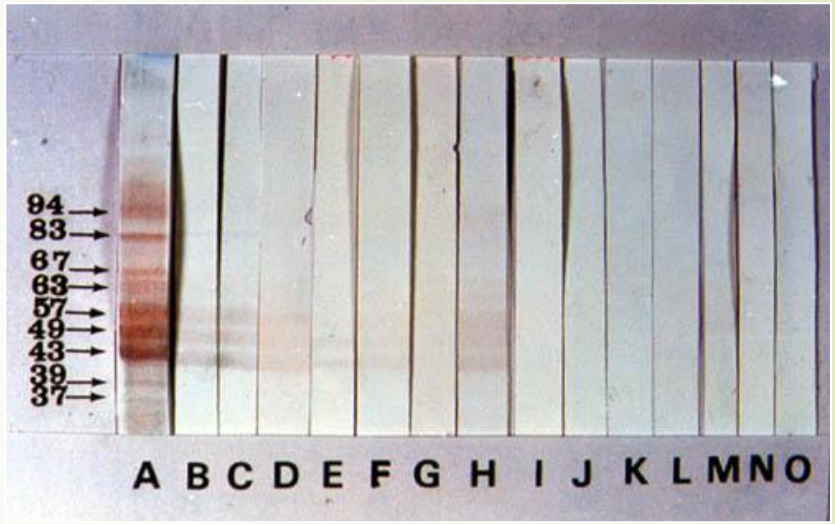
Trichinellosis

พยาธิทริคิเนลลา *Trichinella spiralis*

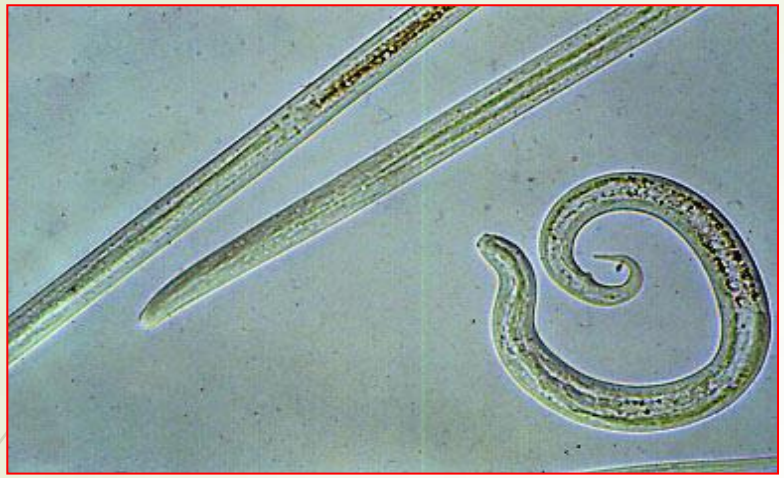


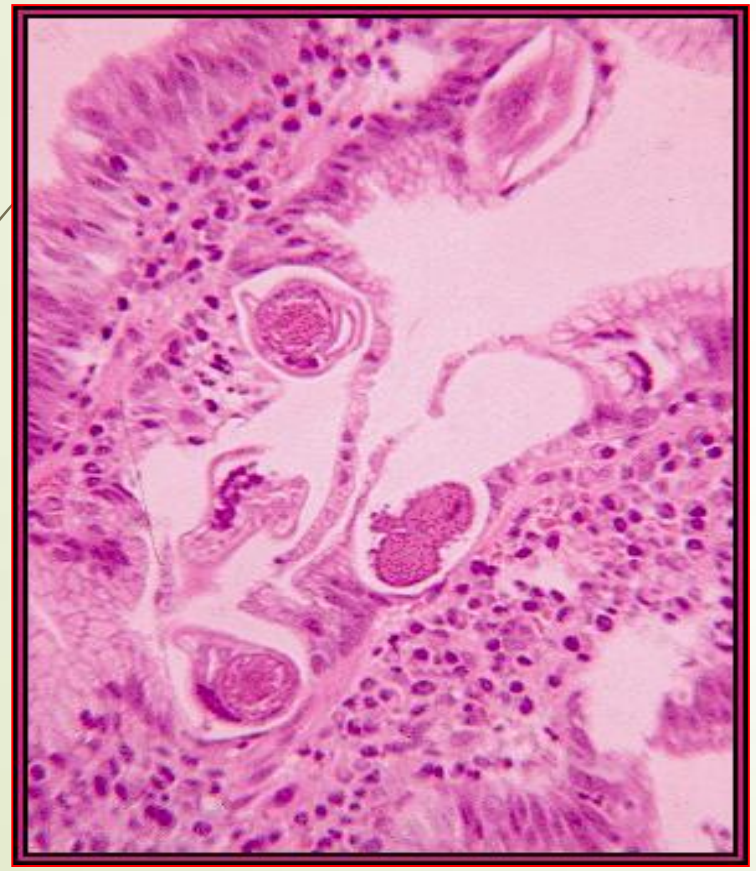


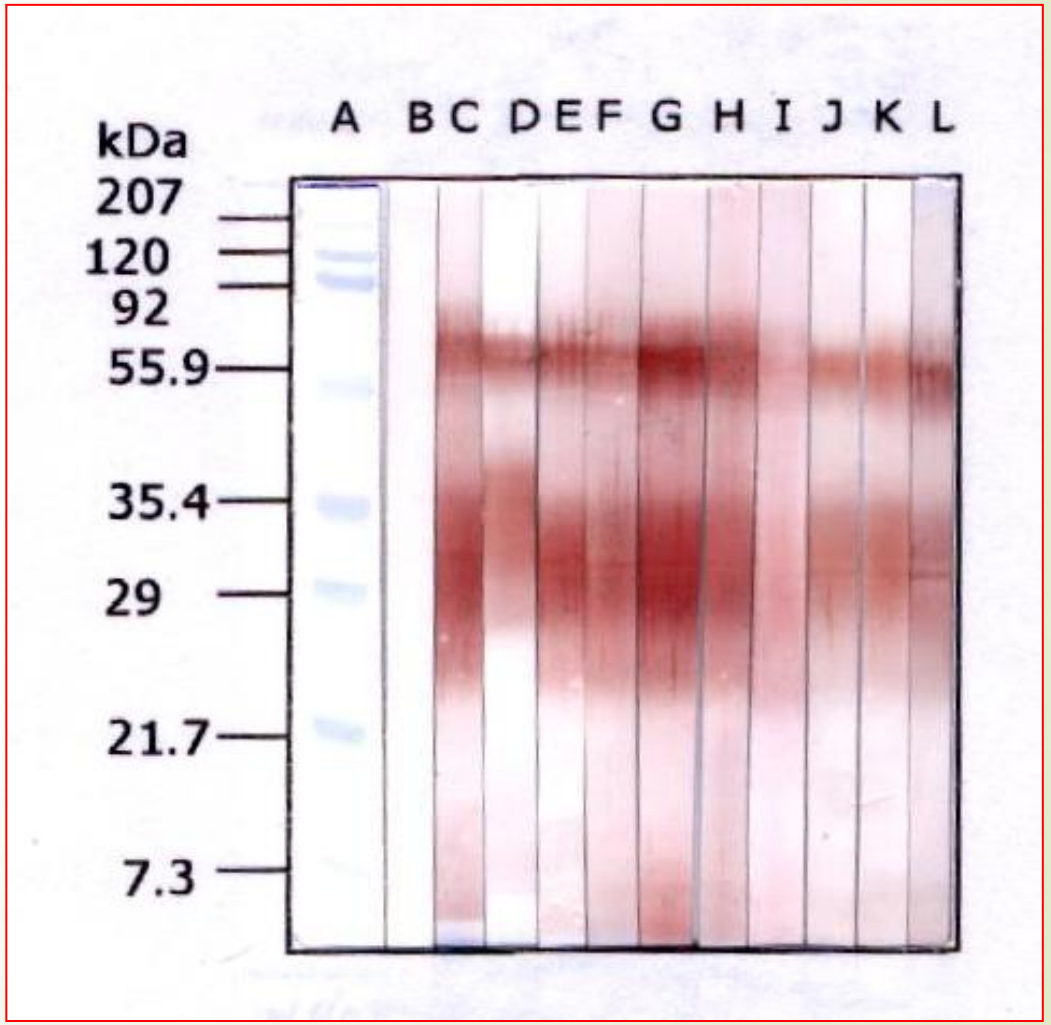




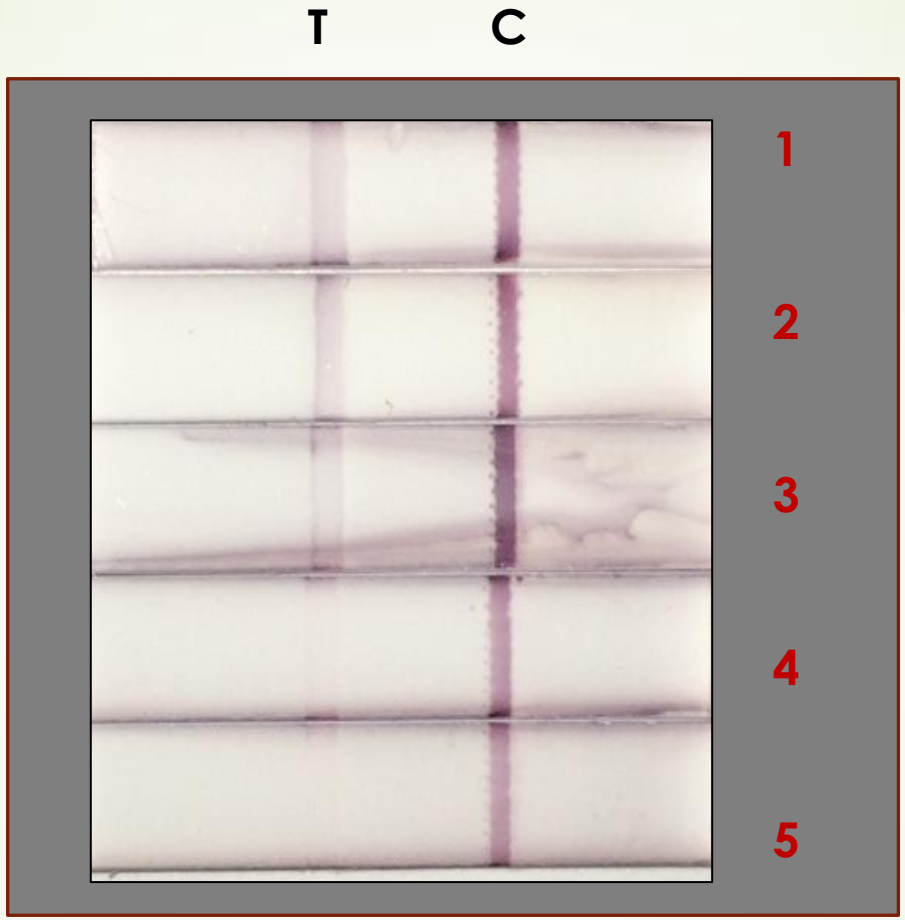
Strongyloidiasis







ชุดตรวจวินิจฉัยโรคฉี่หนู


T
C
1
2
3
4
5

ICT for Scrub Typhus Dx

Scrub Typhus-positive (11-1-103)

Scrub Typhus-positive (11-1-304)

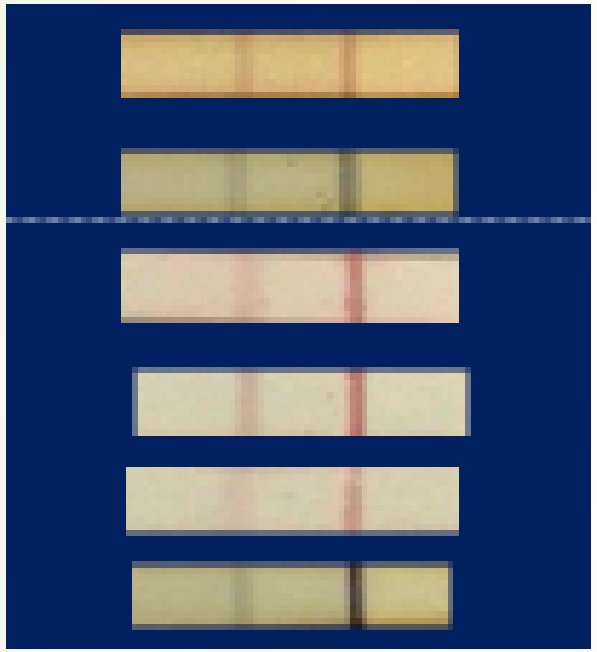
Scrub Typhus-negative (Lepto 0524)

Scrub Typhus-negative (Dengue 0651)

No.	Test		
	IgM/IgG to *O. tsutsugamushi*	Interpretation of the results of 60-kDa-ICT kit test	Results of the 60-kDa-ICT T C
1	IgM Positive IgG Positive (weak)	Positive	
2	IgM Negative IgG Negative	Negative	
3	IgM Negative IgG Negative	Negative	
4	IgM Negative IgG Negative	Negative	
5	IgM Positive IgG Negative	Positive	
6	IgM Positive IgG Negative	Weakly-positive	
7	IgM Negative IgG Positive	Positive	
8	IgM Positive IgG Negative	Positive	
9	IgM Negative IgG Negative	Negative	
10	IgM Positive (weak) IgG Positive (weak)	Positive	
11	IgM Negative IgG Negative	Negative	
12	IgM Negative IgG Negative	Negative	

Immunochromatographic test kit for *Salmonella* detection

T C



ผลบวก มีแถบสีแดงที่ T (Test line) คือมีเชื้อซัลโมเนลลาในตัวอย่างอาหาร

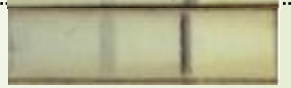
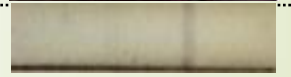
Control line (C) ต้องมีแถบสี

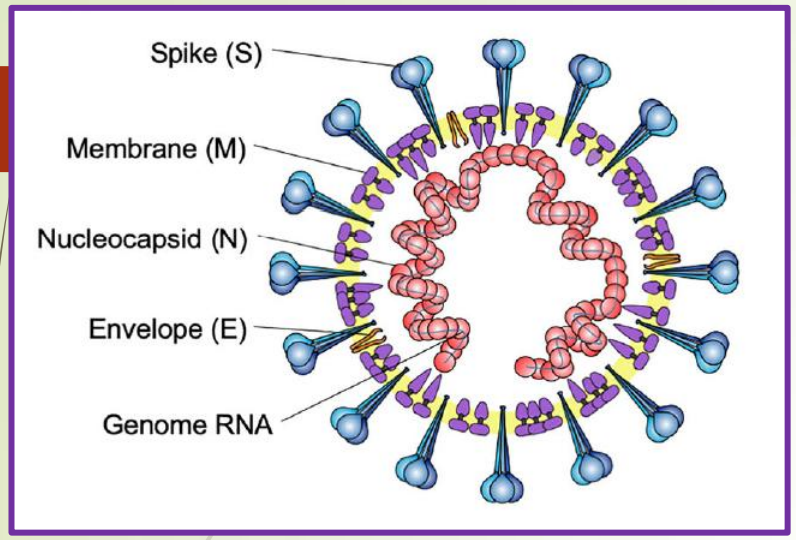
T C

ผลลบ ไม่มีแถบสีแดงที่ T (Test line) คือไม่มีเชื้อซัลโมเนลลาในตัวอย่างอาหาร

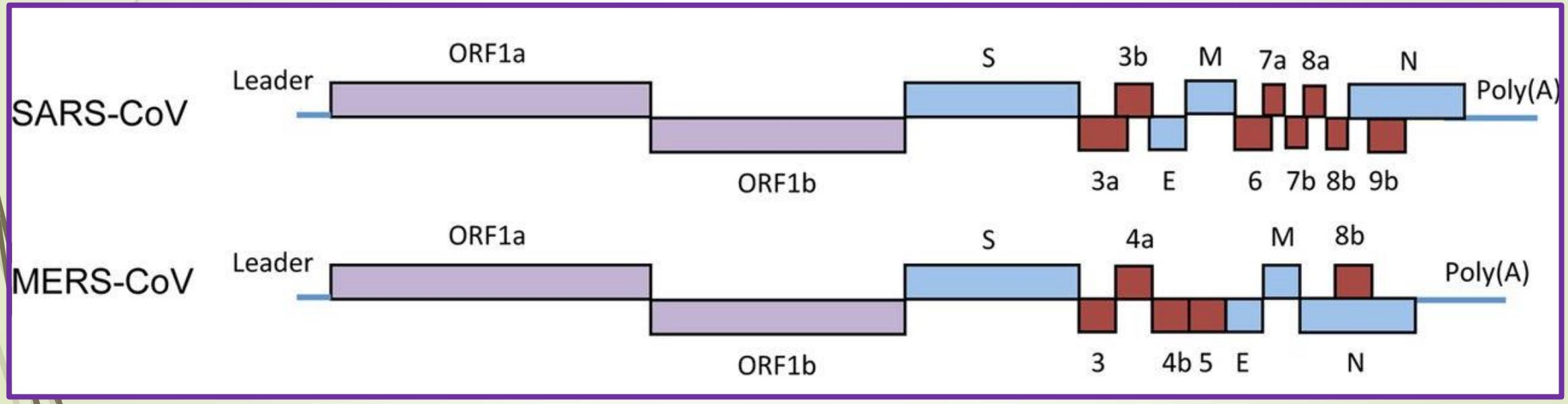
Control line (C) ต้องมีแถบสี

Examples of results: culture method, PCR, and ICT of food samples

Date	Markets	Sample	Result of *Salmonella* culture	Real-time PCR (Ct)	ICT	
8-09-14	Market 1	Pork1	Negative	-(30.64)	Negative	
		Pork2	Negative	-(29.62)	Negative	
		Chicken1	Negative	-(29.64)	Negative	
		Beef1	Positive	+(16.22)	Positive	
29-09-14	Market 2	Pork3	Positive	+(15.75)	Positive	
		Chicken2	Positive	+(16.04)	Positive	
		Chicken3	Positive	+(15.38)	Positive	
		Chicken4	Positive	+(15.57)	Positive	
29-09-14	Market 3	Pork4	Positive	+(15.37)	Positive	
		Beef2	Positive	+(16.69)	Positive	
6-10-14	Market 4	Pork5	Positive	+(18.4)	Positive	
		Pork6	Positive	+(17.79)	Positive	

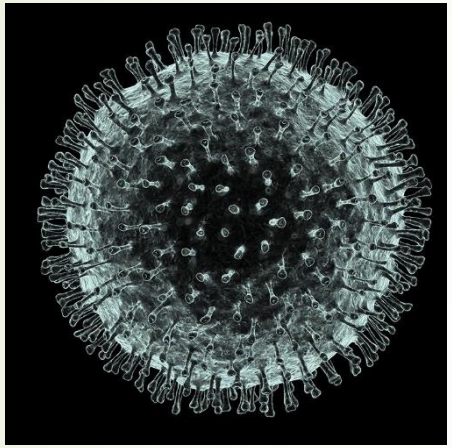


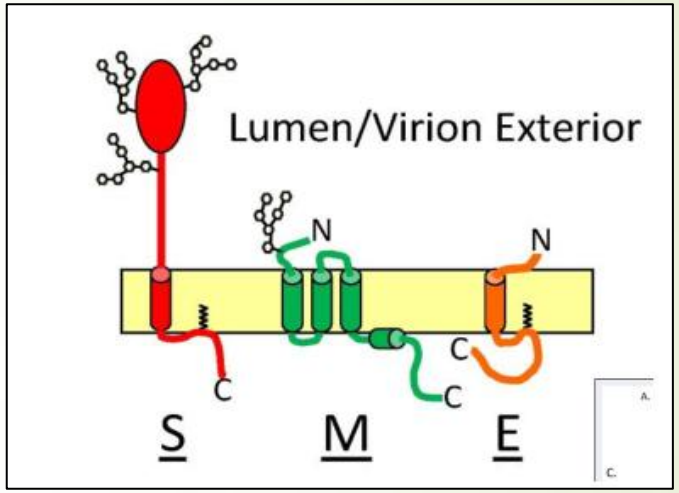
Diagramatic Structure of Coronavirus



Genome Organization of Coronaviruses

Virus-like particles (VLP) vaccine





Conformational stability /

Reserve all native epitopes

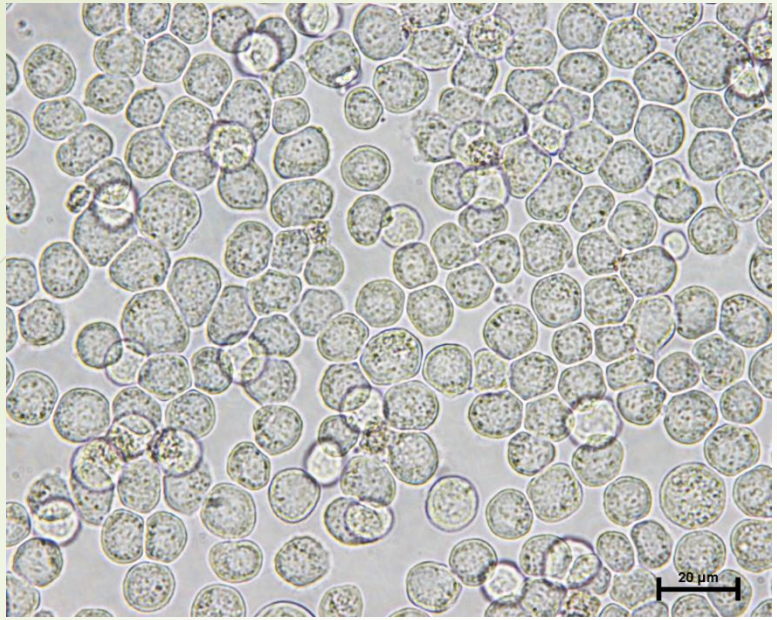
Spike, M and E

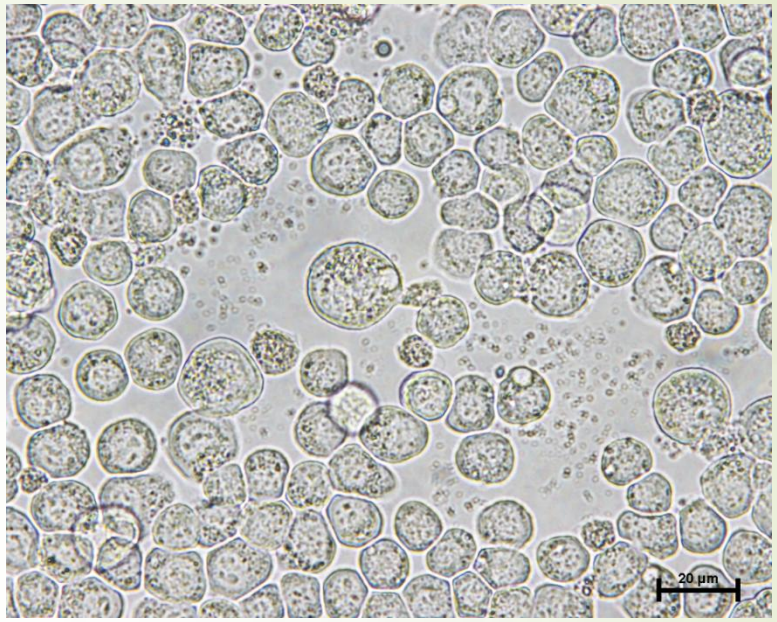
proteins without RNP

Normal

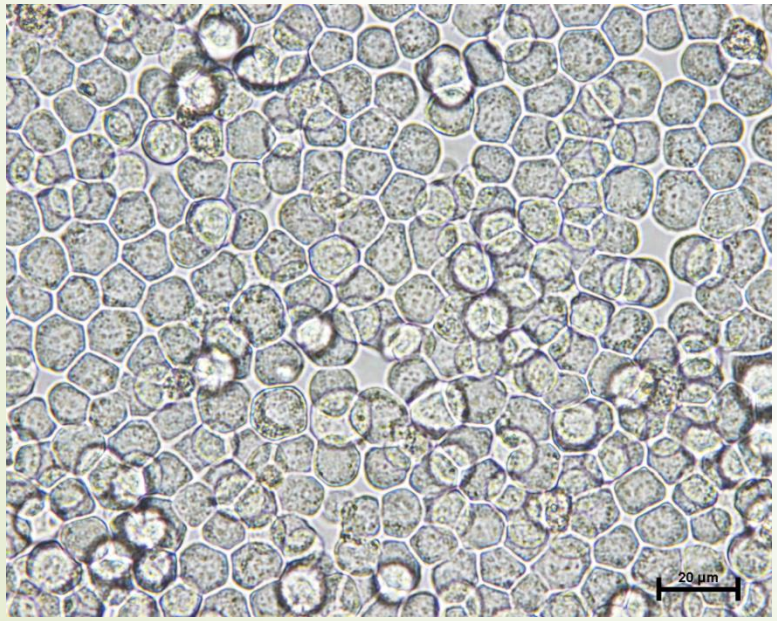
Transfected

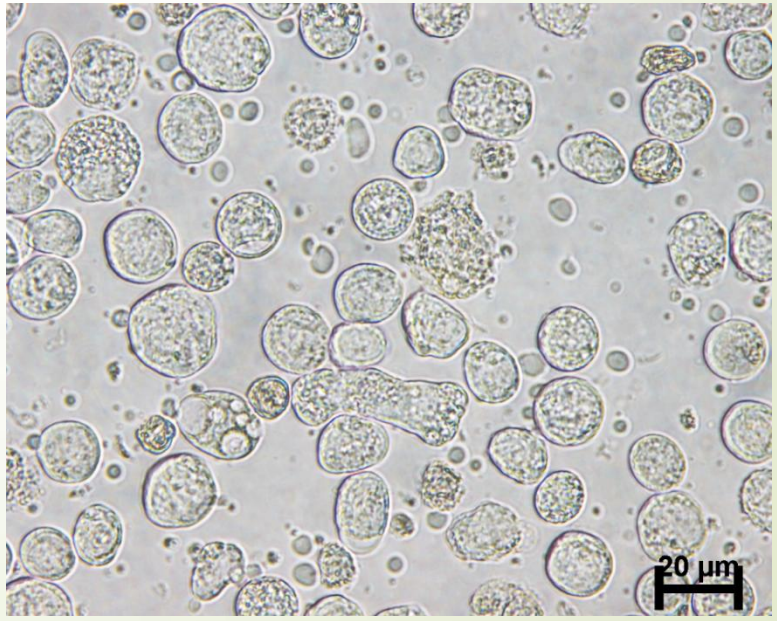
48 hpt

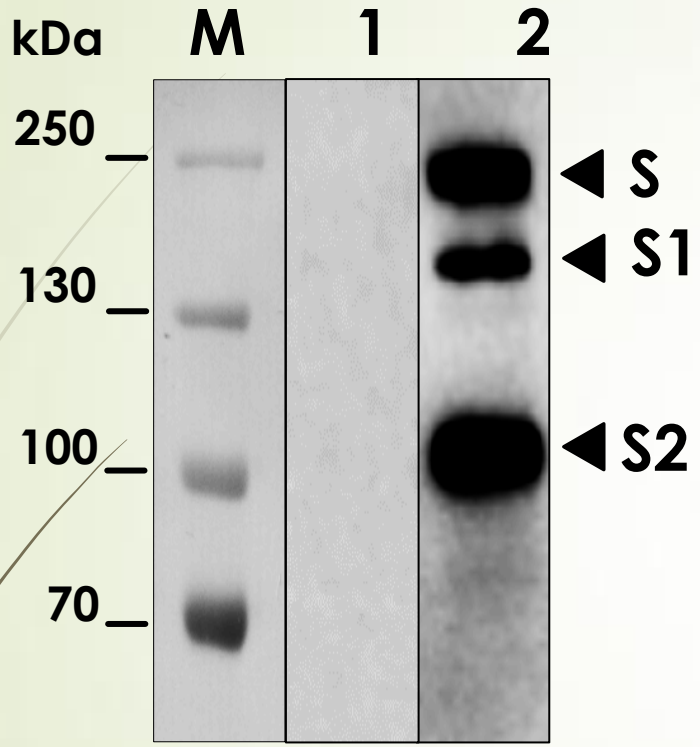




72 hpt



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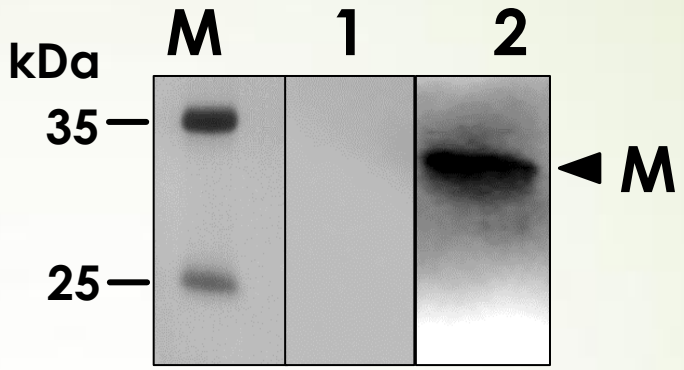
S2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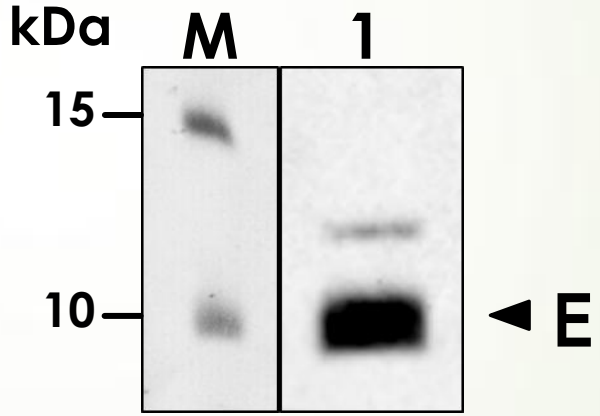
35
25
M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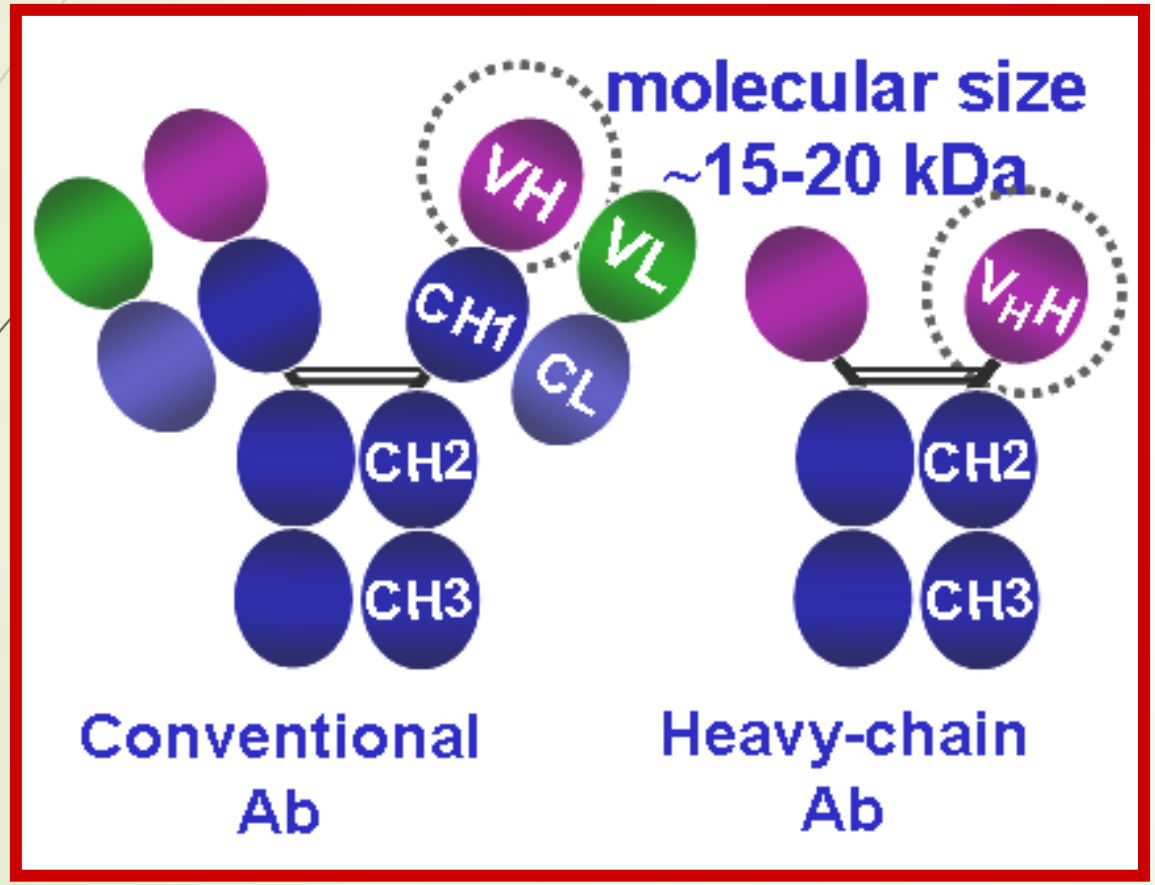
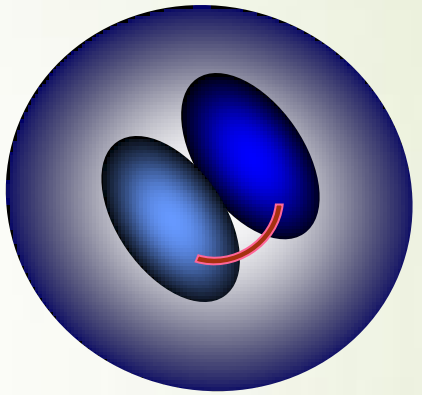
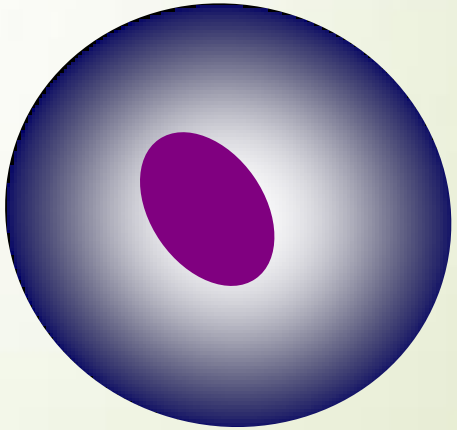
Therapeutic antibodies





scFv



nanobody

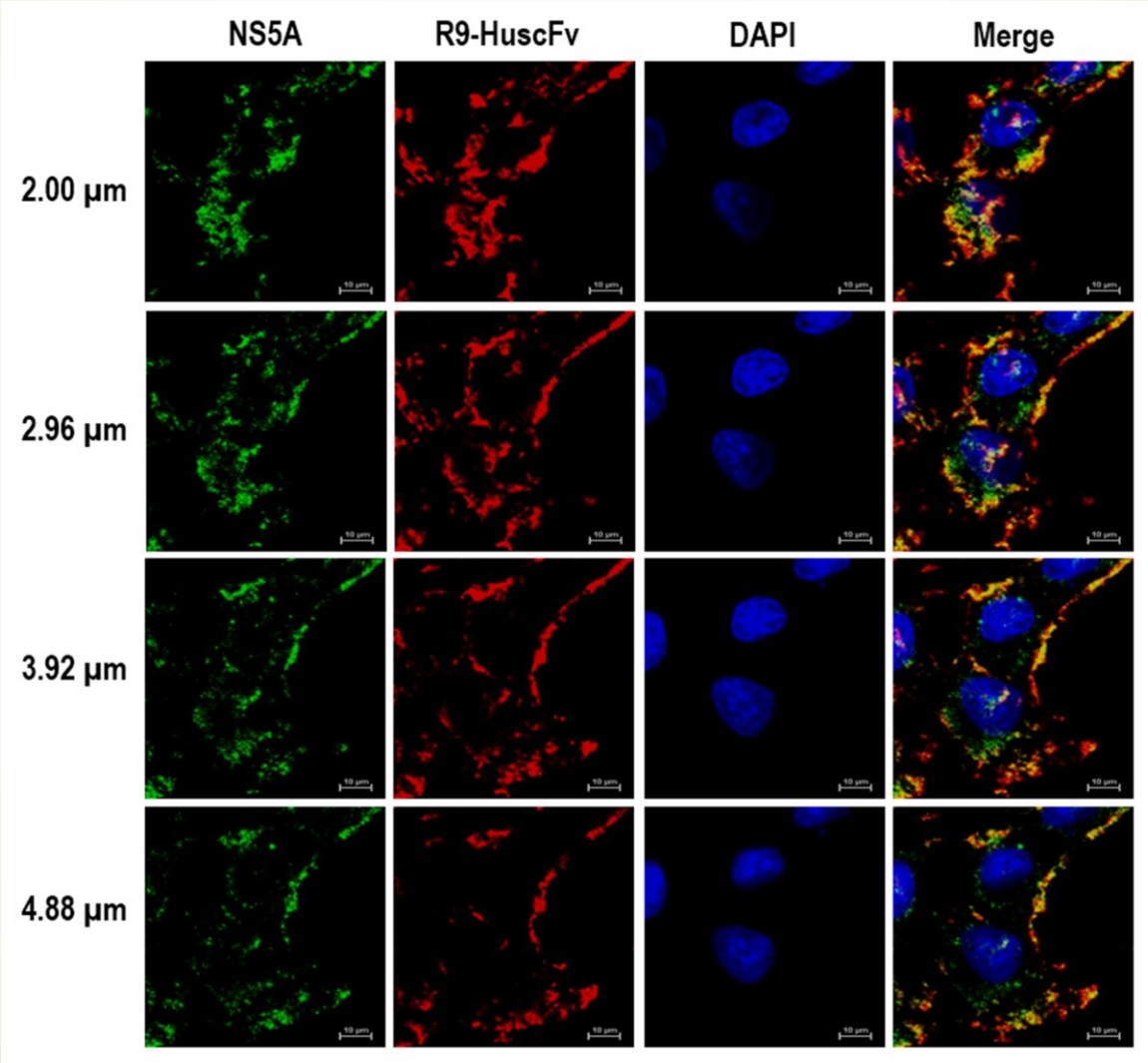
Research Area

1. Engineered fully human antibodies
2. Engineered humanized nanobodies
3. Transbodies (cell penetrating Ab)

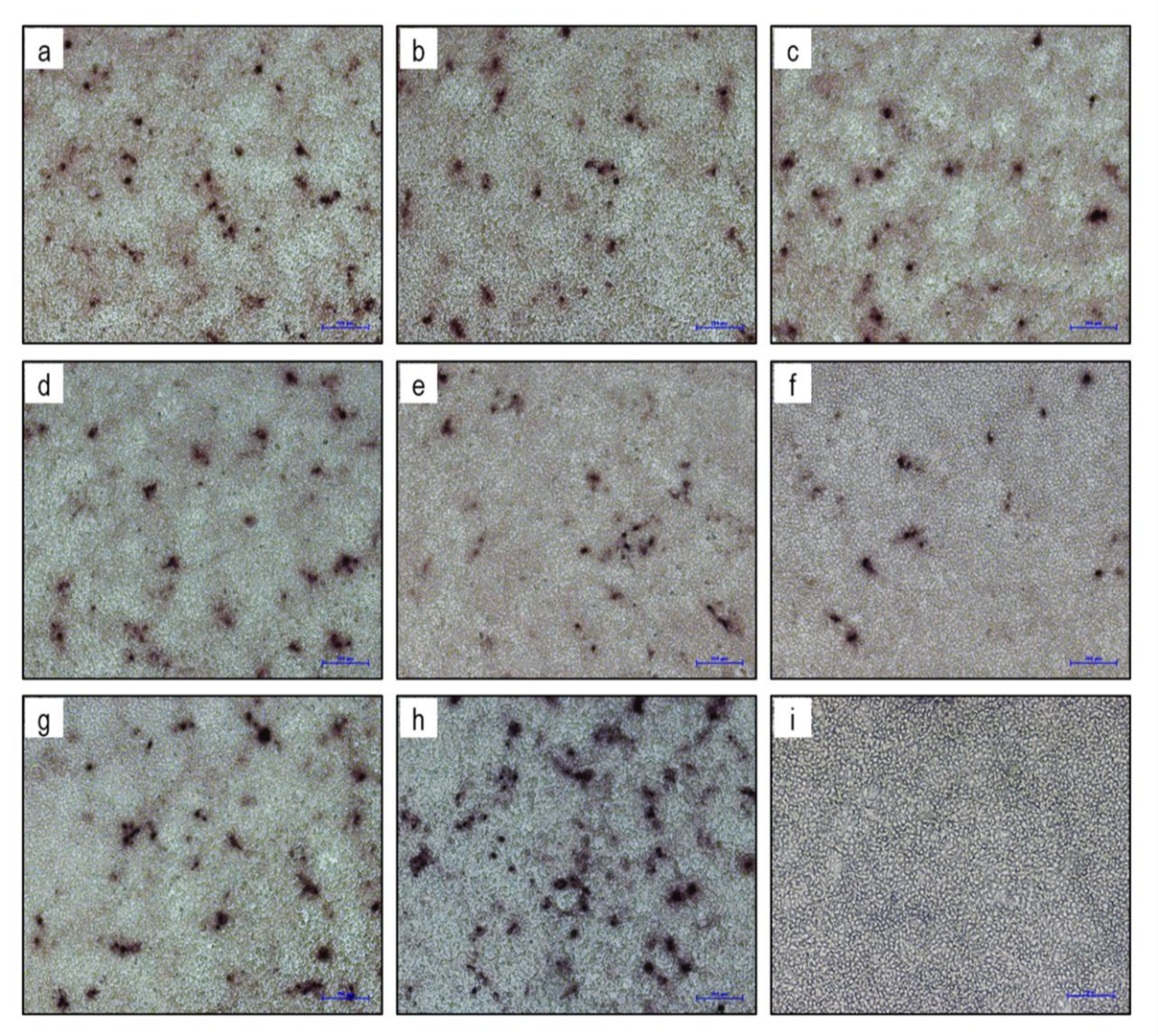
- **Viruses:** Influenza, HCV, EV71, Ebola, porcine epidemic diarrhea (PEDV), SARS-CoV-2, etc.
- **Bacteria/toxins:** tetanus, diphtheria, pertussis, TSST-1, Exotoxin A, botulinum
- **Animal venom/toxin:** cobra neurotoxins, phospholipase A2, kaouthiagin (snake venom metalloproteinase); Tetrodotoxin
- **Cancers:** OX40, PD1/PD-L1, BITE, GD2, Car-T cells, etc.

GMP Production

Cell penetrating antibody (Transbody) against HCV NS5A protein



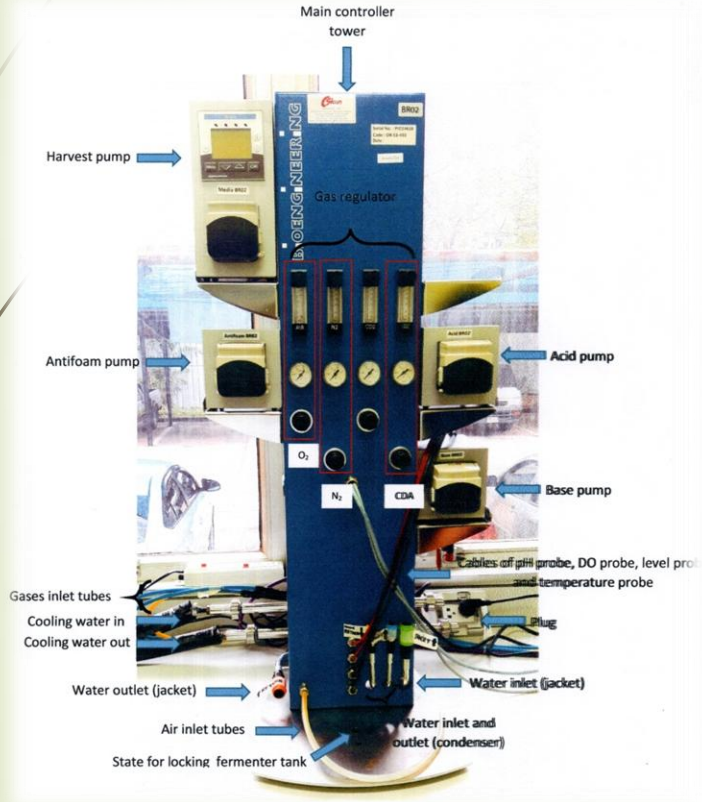
Foci assay for detection of infectious HCV in cells

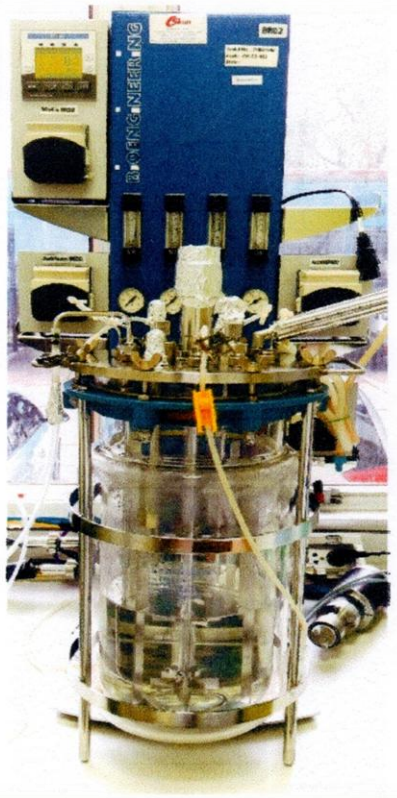


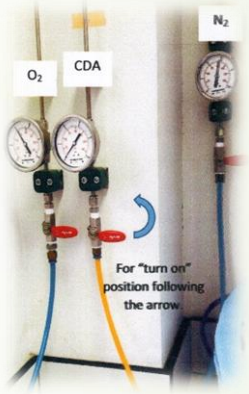
Therapeutic efficacy of transbody to HCV NS5A

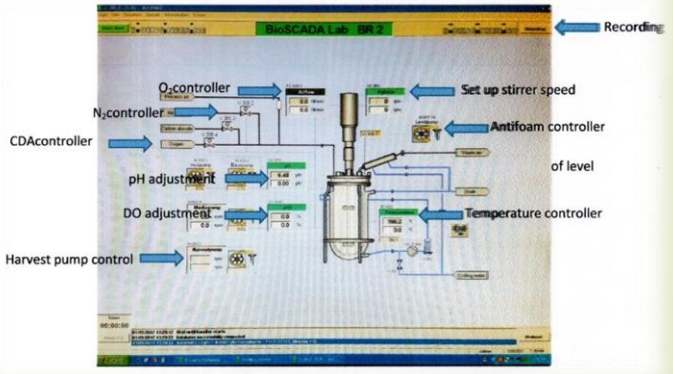
Batch-fermentation of human scFvs in pilot plant (MuBio)

Fermenter, 10-L Bioengineering









That’s all for today

V. cholerae O1 Detection in Rectal Swabs of Diarrheic Patients by Dot-ELISA and Culture Method

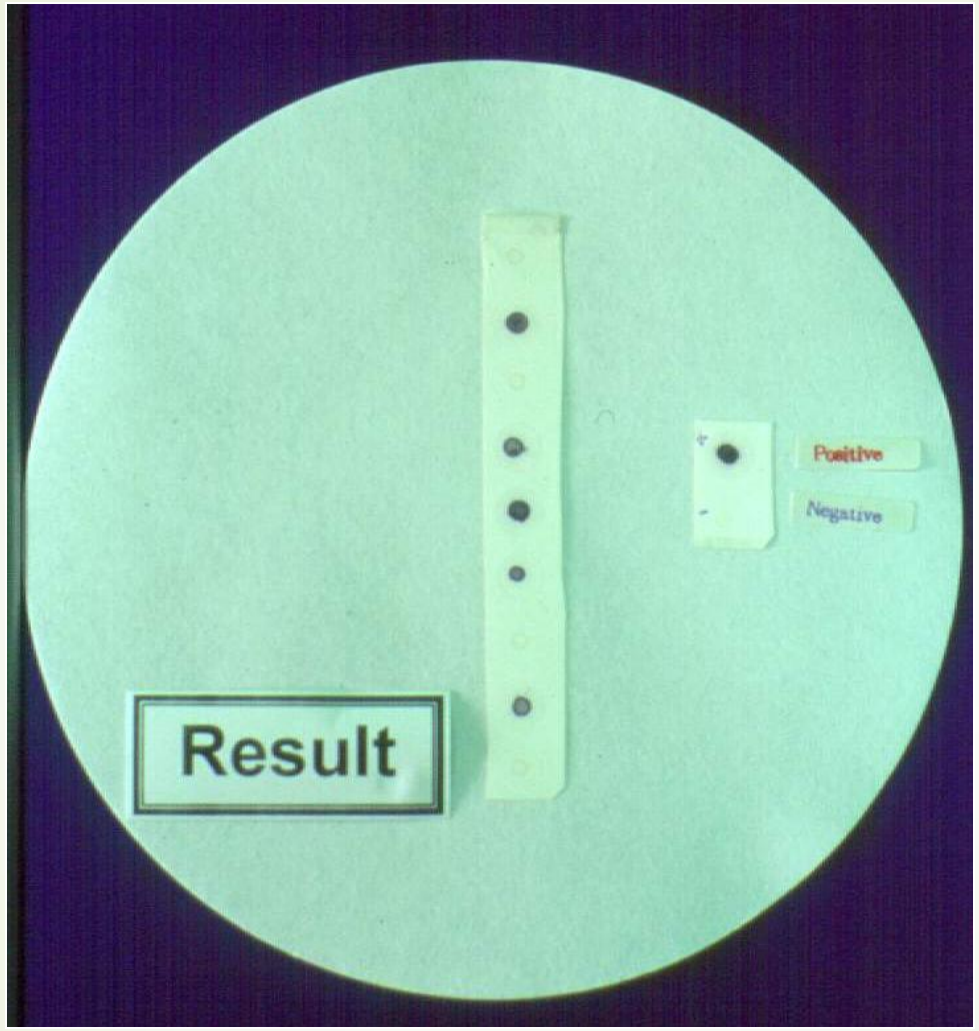
Culture method	Dot-blot ELISA		Total
	Positive	Negative	
• **Culture positive**	14	0	14
• **Culture negative**	0	197	197
• **Total**	14	197	211

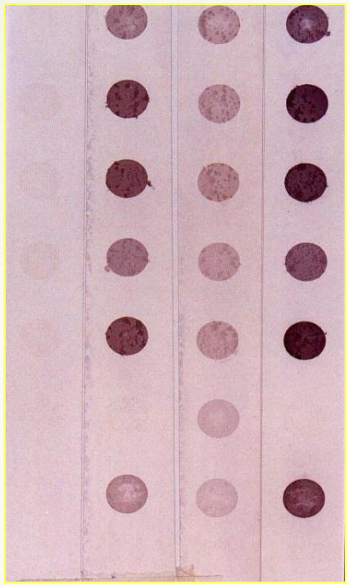
Complete agreement between the two methods

V. cholerae O1 Detection From Rectal Swabs of Case Contacts by Dot-ELISA and Culture Method

	Dot-blot ELISA		Total
	Positive	Negative	
• **Culture positive**	20	1	21
• **Culture negative**	0	394	394
• **Total**	20	395	415

Diagnostic sensitivity 95.2%


Result
+
Positive
-
Negative



L. Autumnalis (Akiyami A)

L. Ballum

L. Djaseman

L. Saigon

L. Wolffi

L. biflexa Patoc

L. Icterohaemorrhagiae

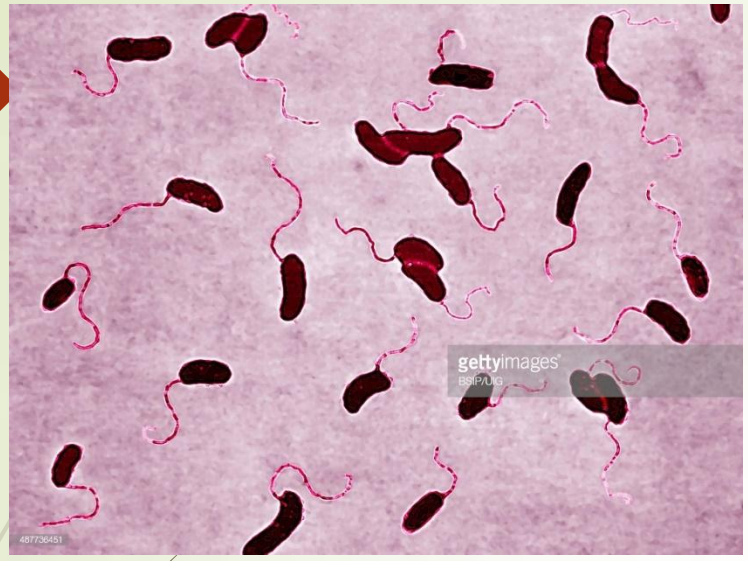
negative

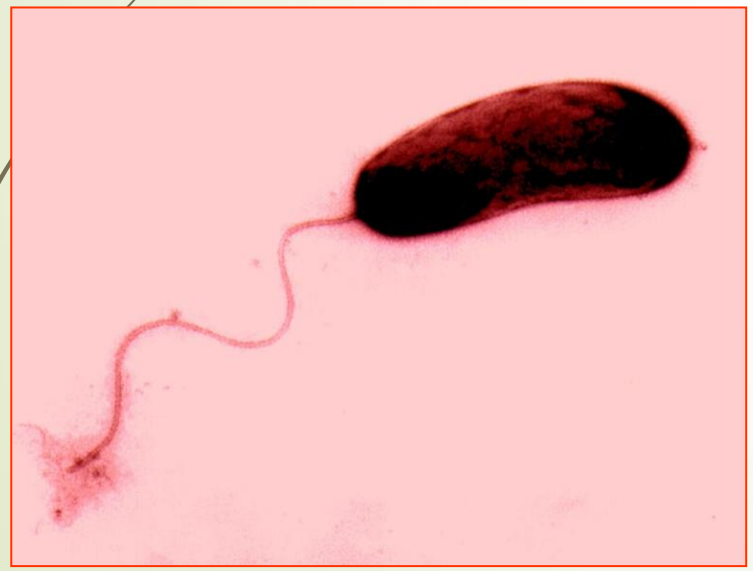
P3x-63-Ag8.653

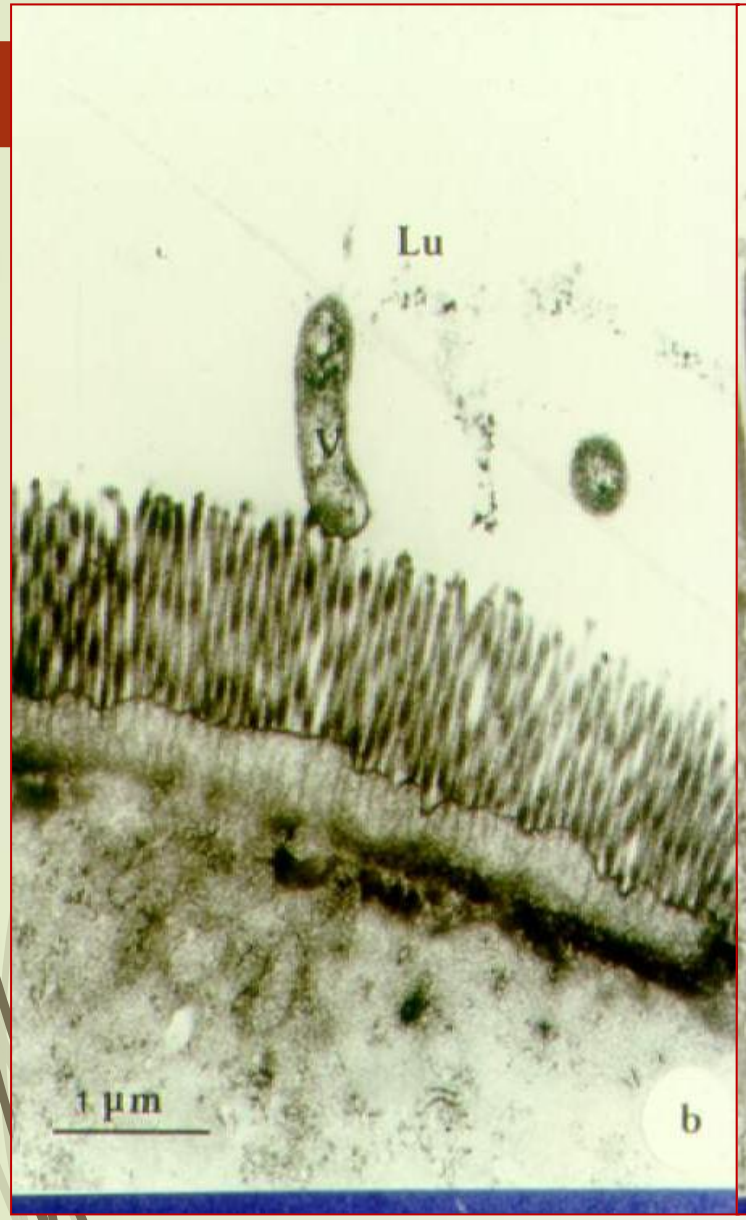
LD5

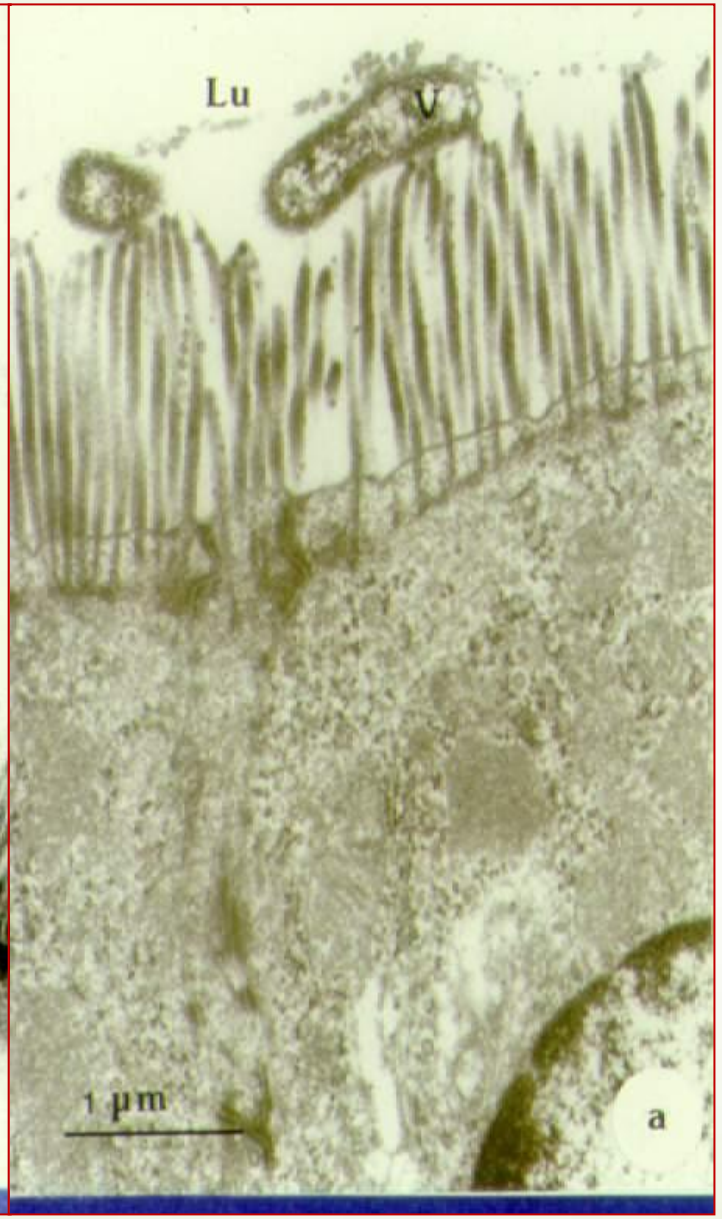
LF9

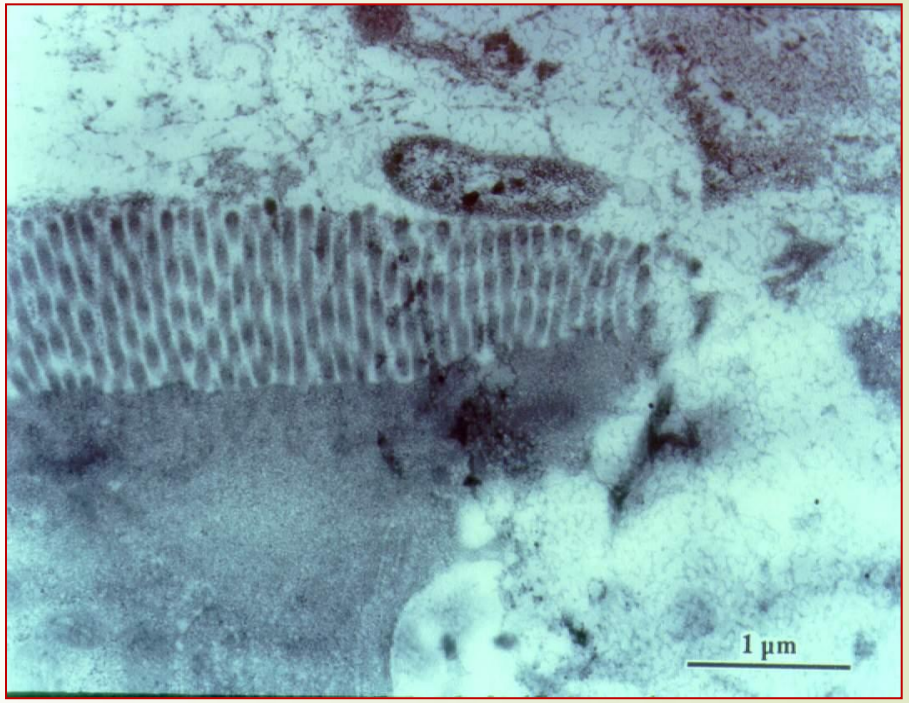
LE1


gettyimages




Lu
V
1 µm
b

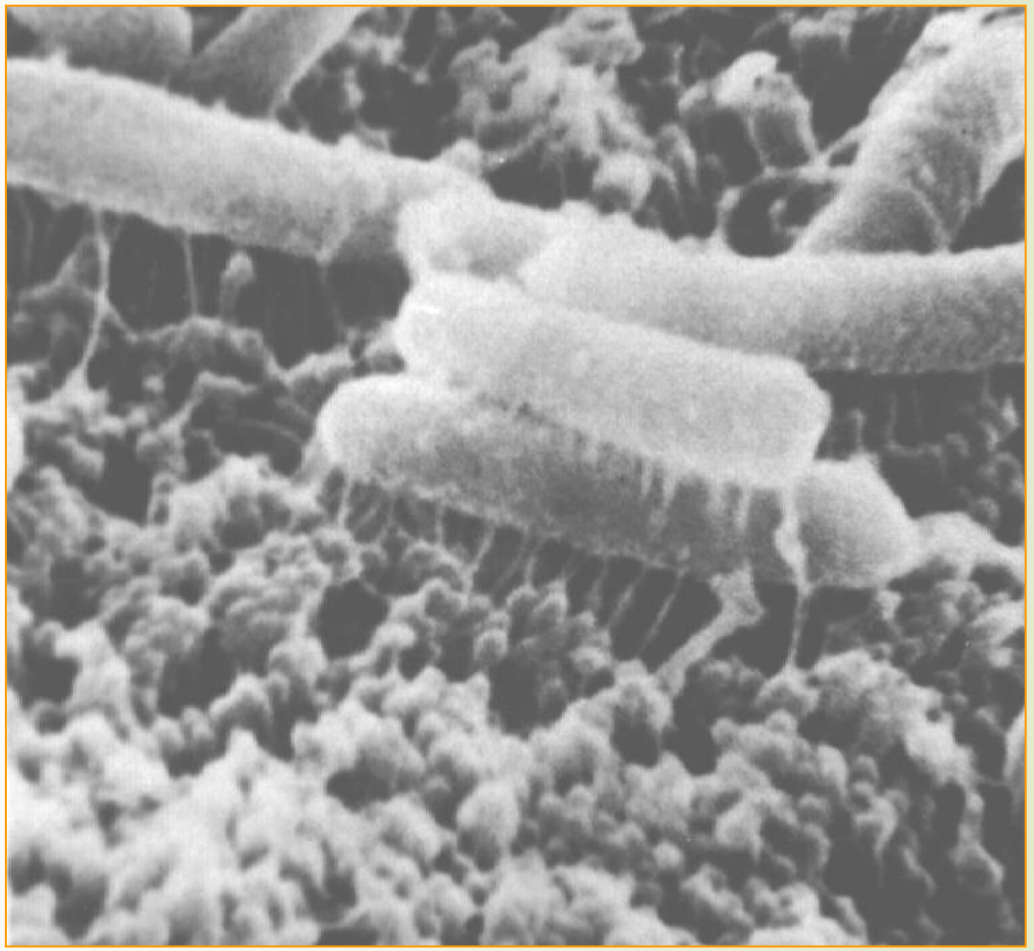

Lu
V
1 µm
a


1 µm

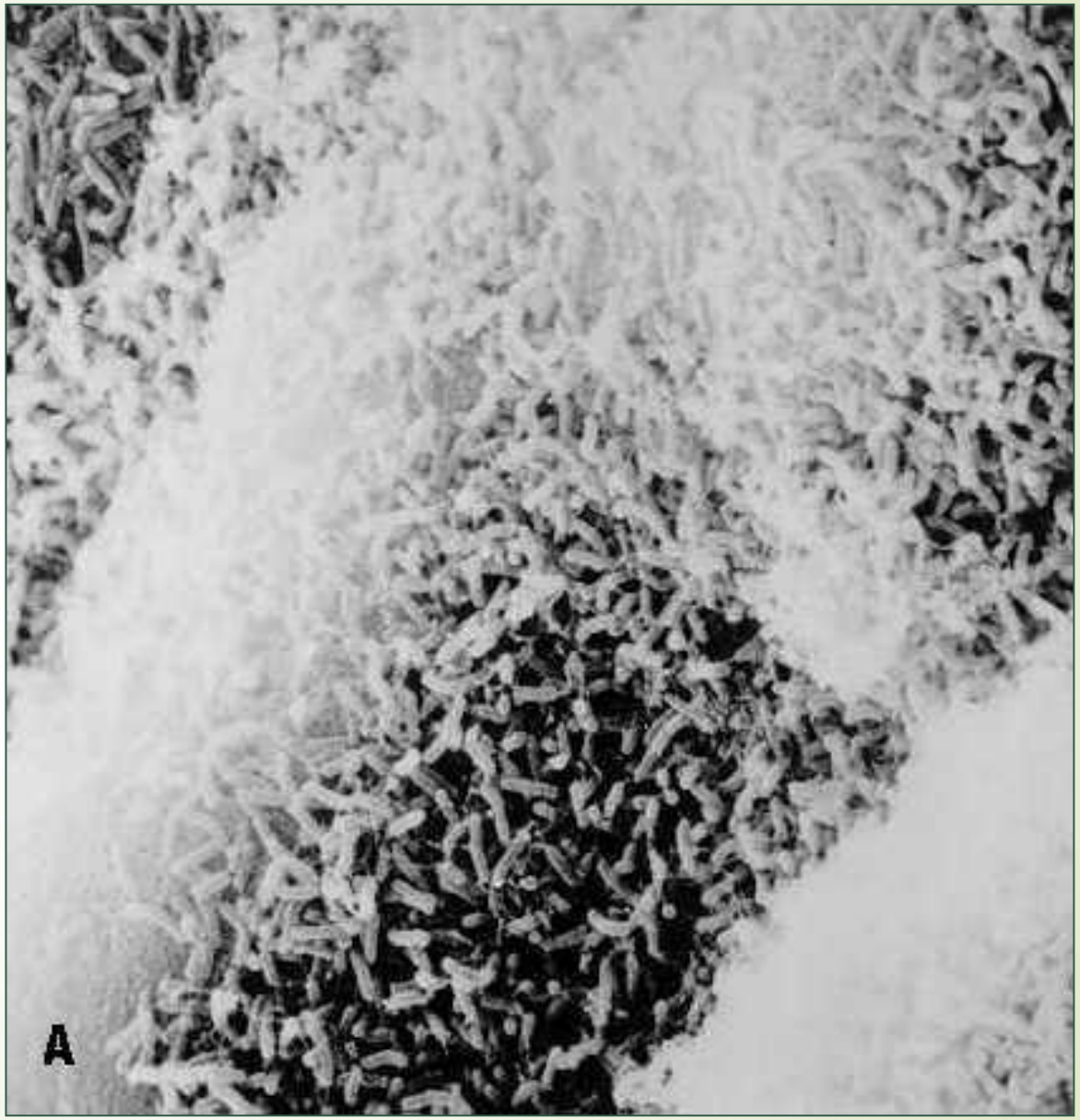

B


C

Vibrio cholerae attachment to microvillus of intestinal epithelial cell *via* a toxin-co-regulated pilus (TCP)



V. cholerae attachment and colonization in small intestine



Infect Immun 14:527, 1976

Subunit oral cholera vaccine

1. Toxin co-regulated pili
2. Lipopolysaccharide

} Prevent attachment

3. B subunit of cholera toxin → Neutralize toxin

↑ Liposome as adjuvant