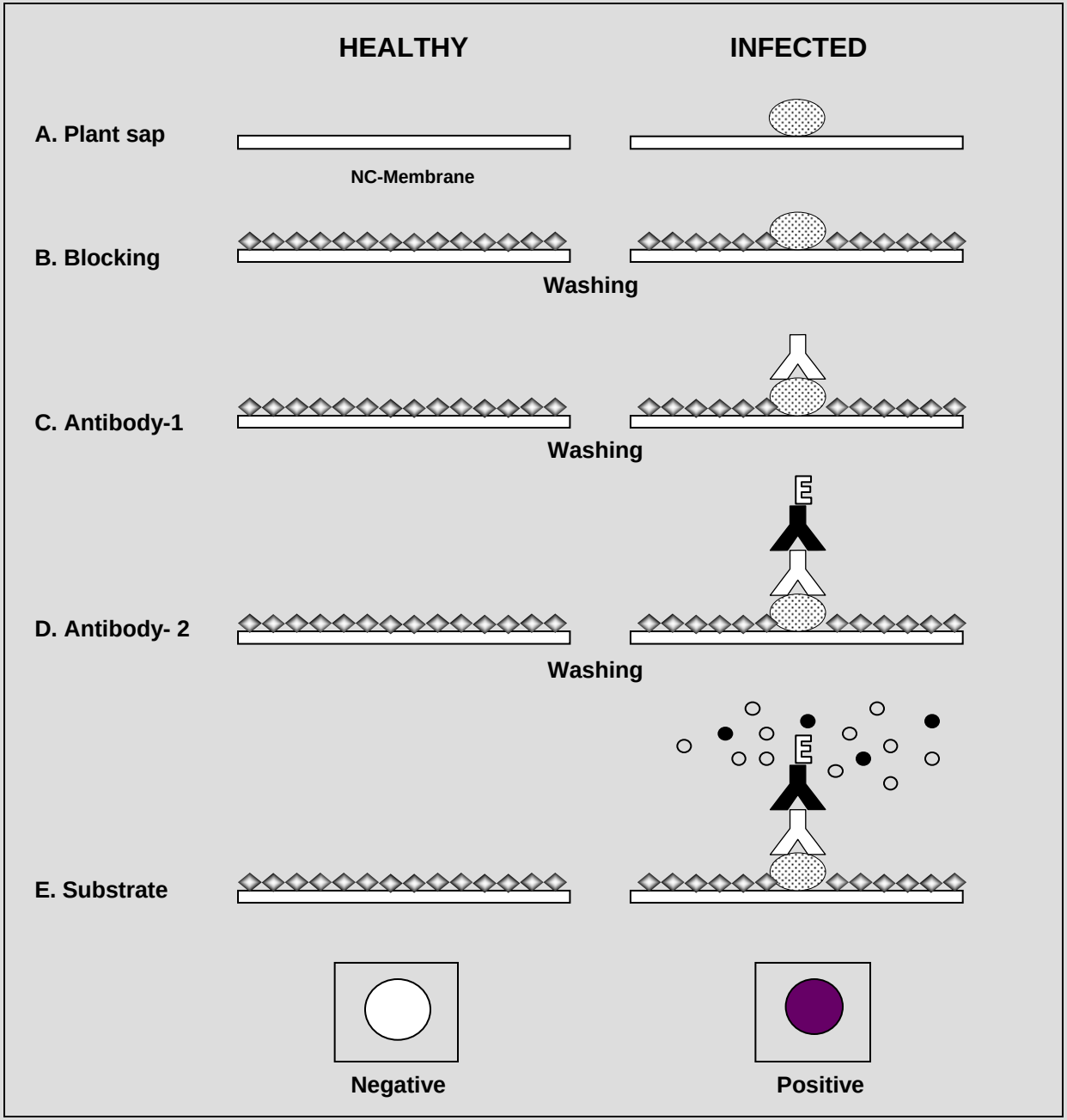
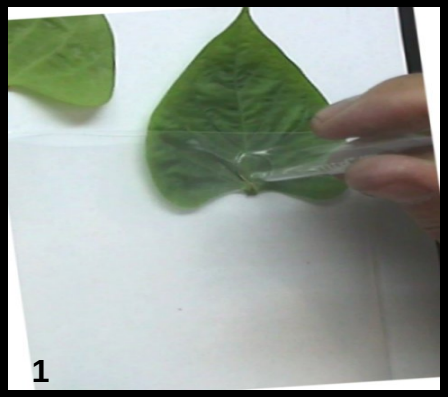
NCM-ELISA

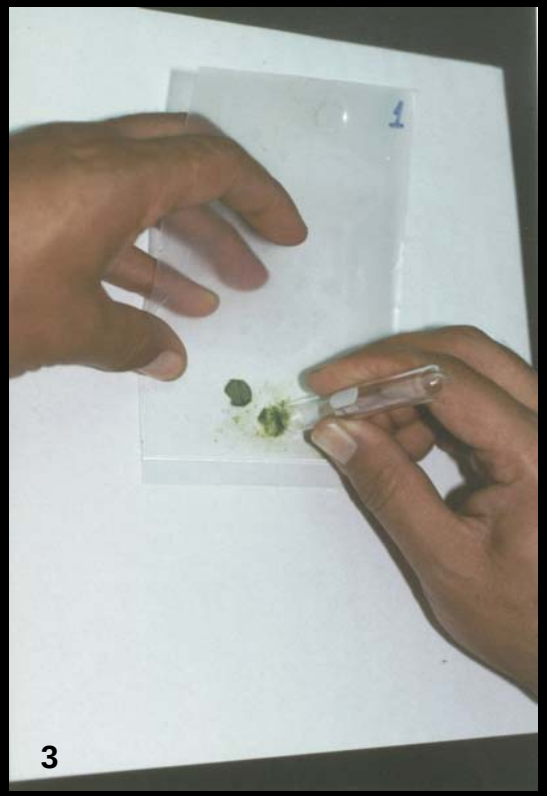
Steps followed in NCM-ELISA



Sample preparation



Cutting leaf disks



Grinding leaf disks

(1 ml of extraction buffer per each leaf disk)

Extraction buffer

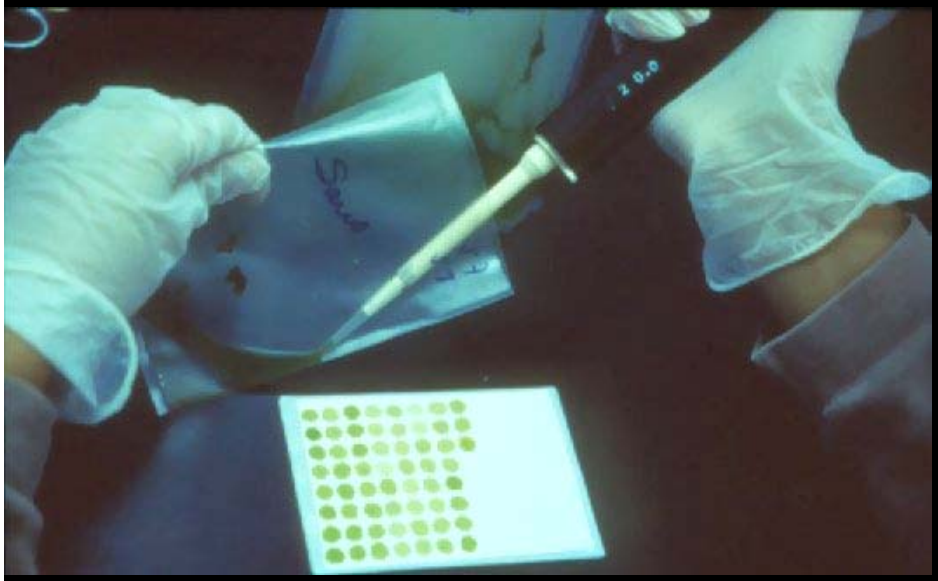
Sodium sulfite (Na_2SO_3)	0.2g (0.2%)
TBS	100 ml

Tris Buffer Saline (TBS), pH 7.5 (2,000 ml)

Tris base	4.84 g	(0.02M)
NaCl	58.44 g	(0.5 M)

Dissolve in 1,990 ml distilled water and adjust pH to 7.5 with HCl (5 N). Add distilled water to 2,000 ml final volume.

Application of samples to the nitrocellulose membrane



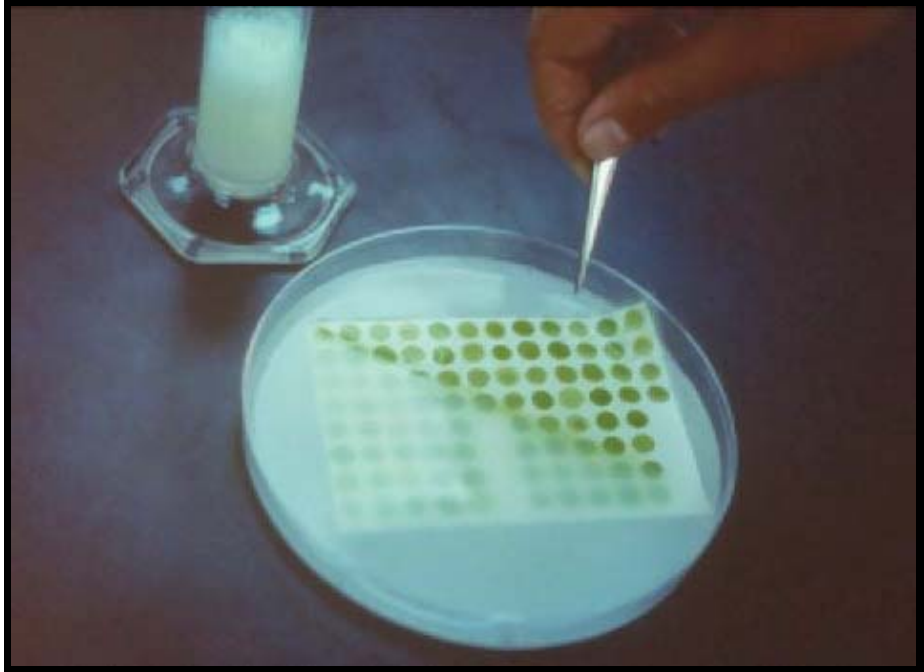
Method 1

Spot 17 ul sap onto each square of the nitrocellulose membrane included in the kit

Method 2

Using a dot-blotting apparatus connected to a vacuum pump (at 210 mm Hg) add 30-50 ul sap

Blocking the nitrocellulose membrane

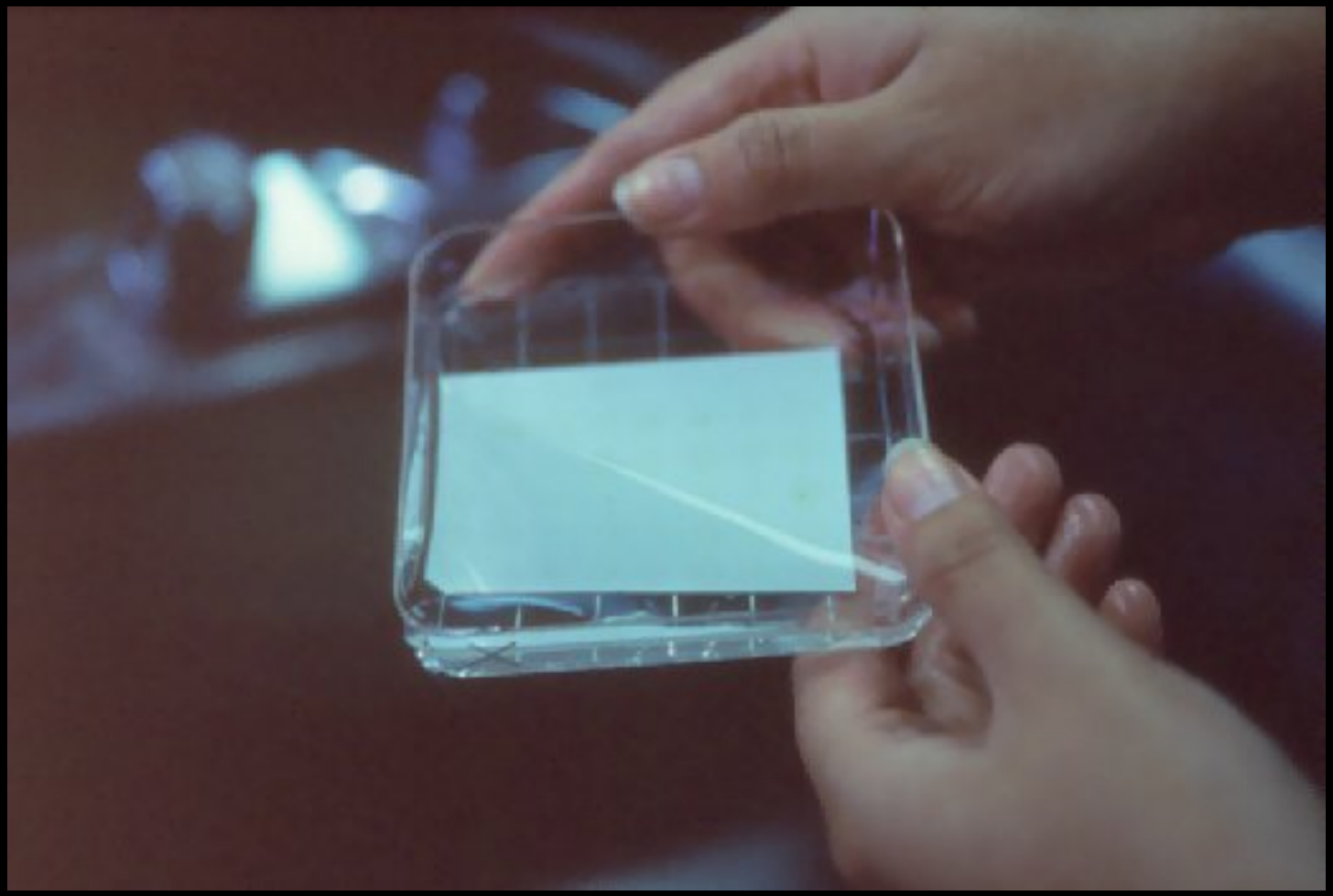


Blocking buffer solution

Milk powder (2%)
Triton X-100 (2%)
TBS

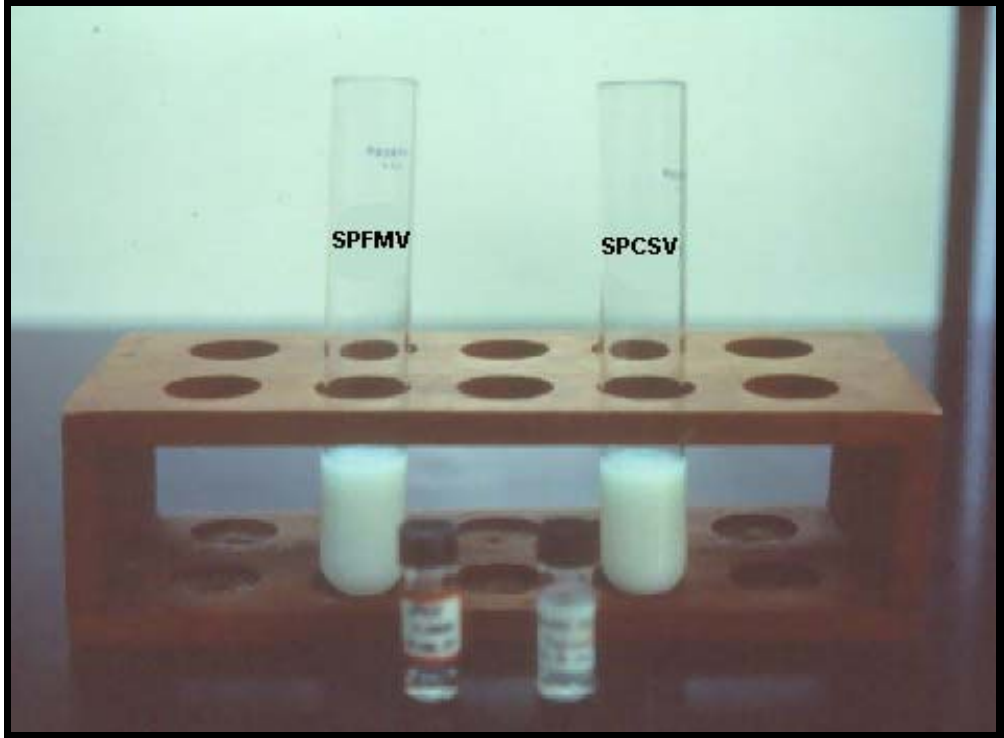
Incubate for 1 hour

Washing step



With TBS (one time, quickly)

Antibody-1
(virus - specific)



<u>Antibody buffer solution</u>

Milk powder (2%)
TBS **(30 ml / membrane)**
Antibody-1 **(according to instructions in bottle)**

Incubate overnight

Washing step

With T-TBS (TBS containing 0.05% Tween-20)
(wash four times, 3 minutes each)

Antibody-2

(anti - antibody conjugated to enzyme)

Antibody buffer solution

Milk powder (2%)
TBS **(30 ml / membrane)**
Antibody-2 **(according to instructions in bottle)**

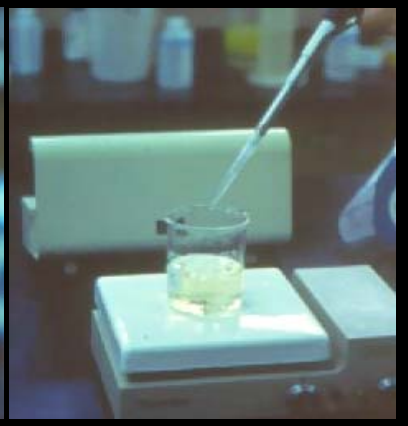
Incubate for 1 hour

Washing step

With T-TBS (TBS containing 0.05% Tween-20)
(wash four times, 3 minutes each)

Preparation of substrate solution

+



NBT

(Nitro blue tetrazolium)

BCIP

(5 Bromo-4-chloro-3-indolyl phosphate)

Substrate solution (per membrane)

NBT	3.0 mg
BCIP	1.5 mg
Substrate buffer	30 ml

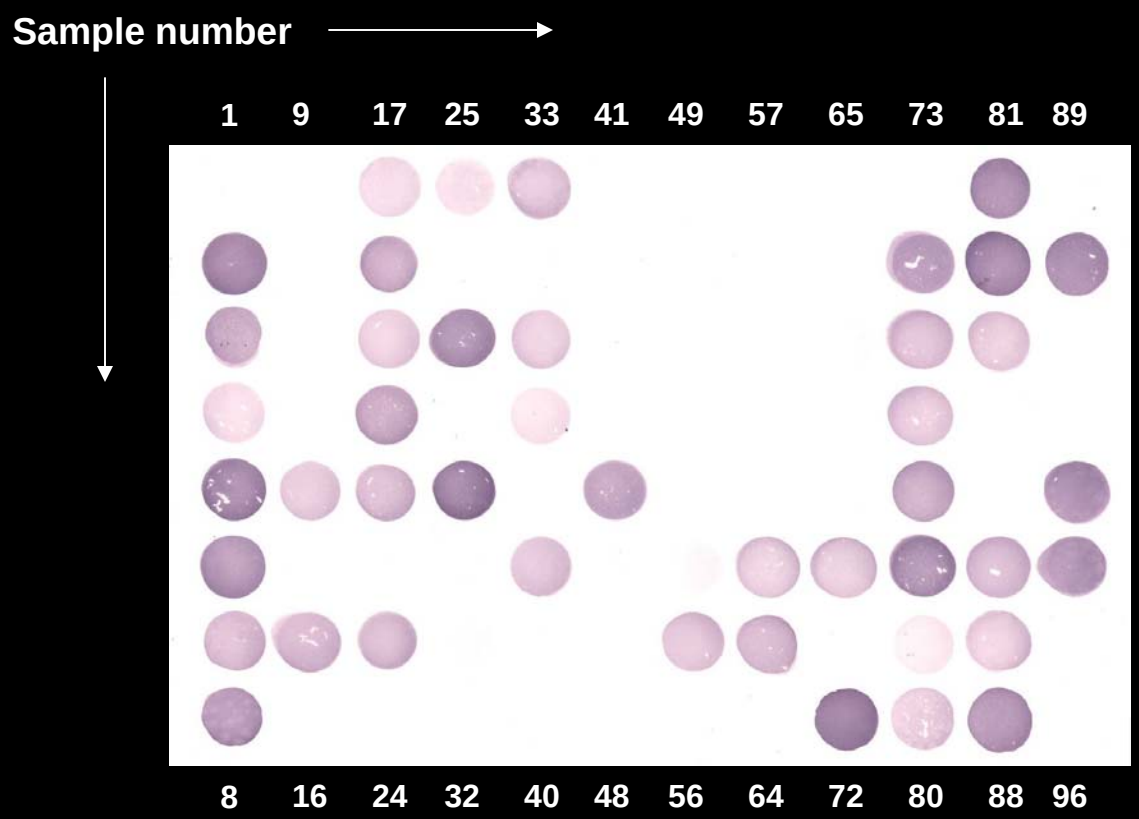
Substrate buffer, pH 9.5

Tris base	0.1 M
NaCl	0.1 M
$MgCl_2$	0.005 M (= 5 mM)

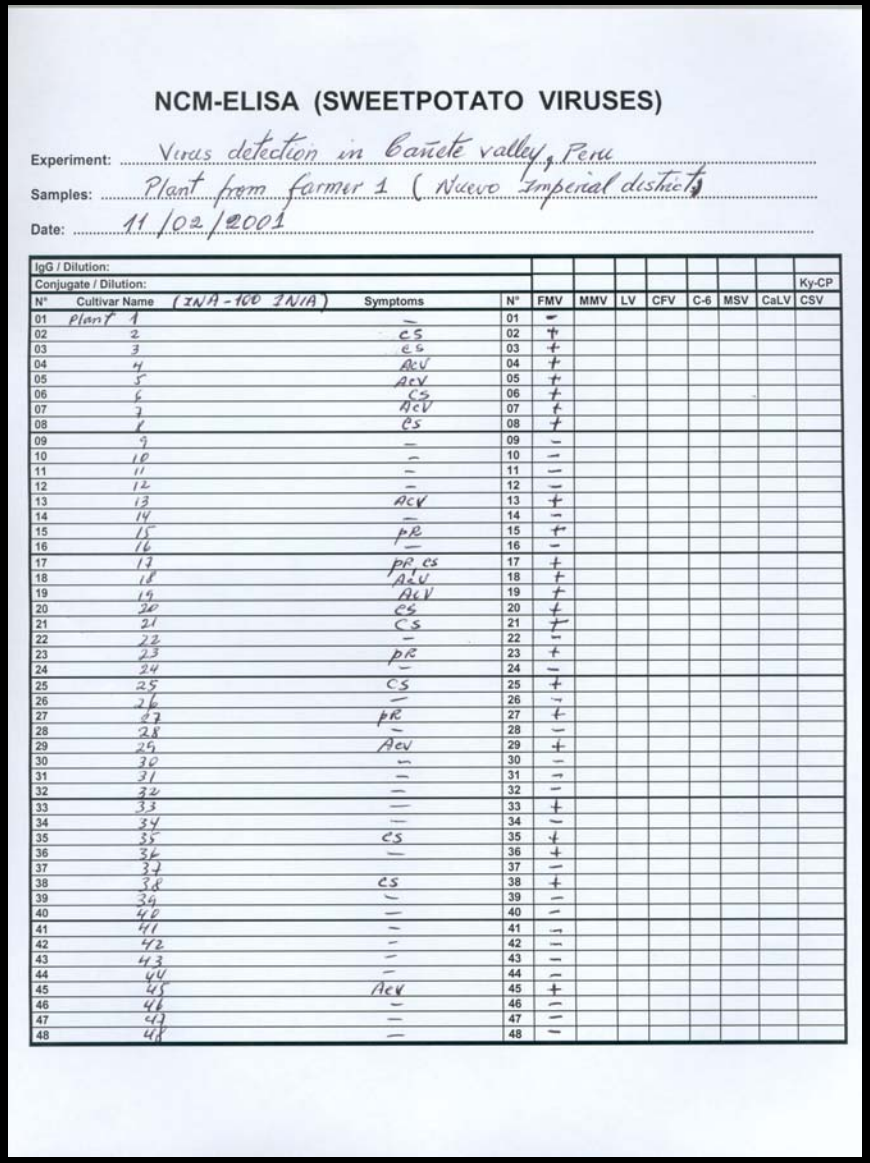
Development of the reaction

Incubate for 30 to 60 minutes

Reading results



Recording results



NCM-ELISA (SWEETPOTATO VIRUSES)

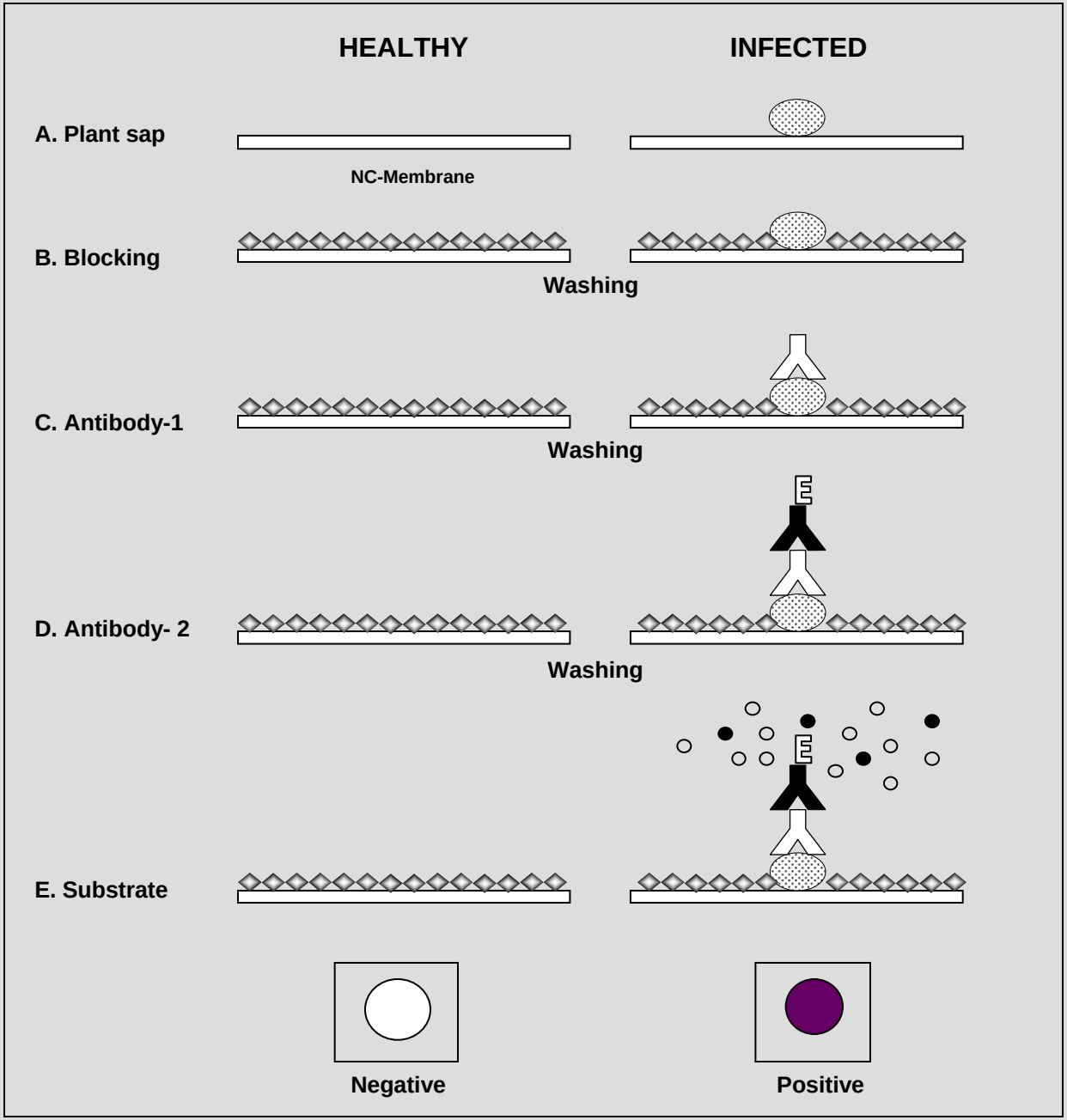
Experiment: Virus detection in Cañete valley, Peru

Samples: Plant from farmer 1 (Nuevo Imperial district)

Date: 11/02/2001

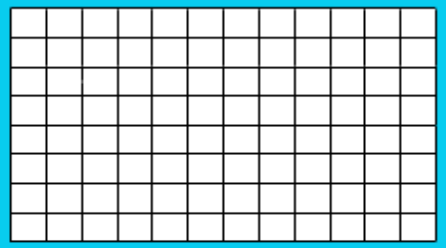
IgG / Dilution:											
Conjugate / Dilution:											Ky-CP
N°	Cultivar Name (INIA-100 INIA)	Symptoms	N°	FMV	MMV	LV	CFV	C-6	MSV	CaLV	CSV
01	Plant 1	–	01	–							
02	2	CS	02	+							
03	3	CS	03	+							
04	4	AcV	04	+							
05	5	AcV	05	+							
06	6	CS	06	+							
07	7	AcV	07	+							
08	8	CS	08	+							
09	9	–	09	–							
10	10	–	10	–							
11	11	–	11	–							
12	12	–	12	–							
13	13	AcV	13	+							
14	14	–	14	–							
15	15	pR	15	+							
16	16	–	16	–							
17	17	pR CS	17	+							
18	18	AcV	18	+							
19	19	AcV	19	+							
20	20	CS	20	+							
21	21	CS	21	+							
22	22	–	22	–							
23	23	pR	23	+							
24	24	–	24	–							
25	25	CS	25	+							
26	26	–	26	–							
27	27	pR	27	+							
28	28	–	28	–							
29	29	AcV	29	+							
30	30	–	30	–							
31	31	–	31	–							
32	32	–	32	–							
33	33	–	33	+							
34	34	–	34	–							
35	35	CS	35	+							
36	36	–	36	+							
37	37		37	–							
38	38	CS	38	+							
39	39	–	39	–							
40	40	–	40	–							
41	41	–	41	–							
42	42	–	42	–							
43	43	–	43	–							
44	44	–	44	–							
45	45	AcV	45	+							
46	46	–	46	–							
47	47	–	47	–							
48	48	–	48	–							

Steps followed in NCM-ELISA



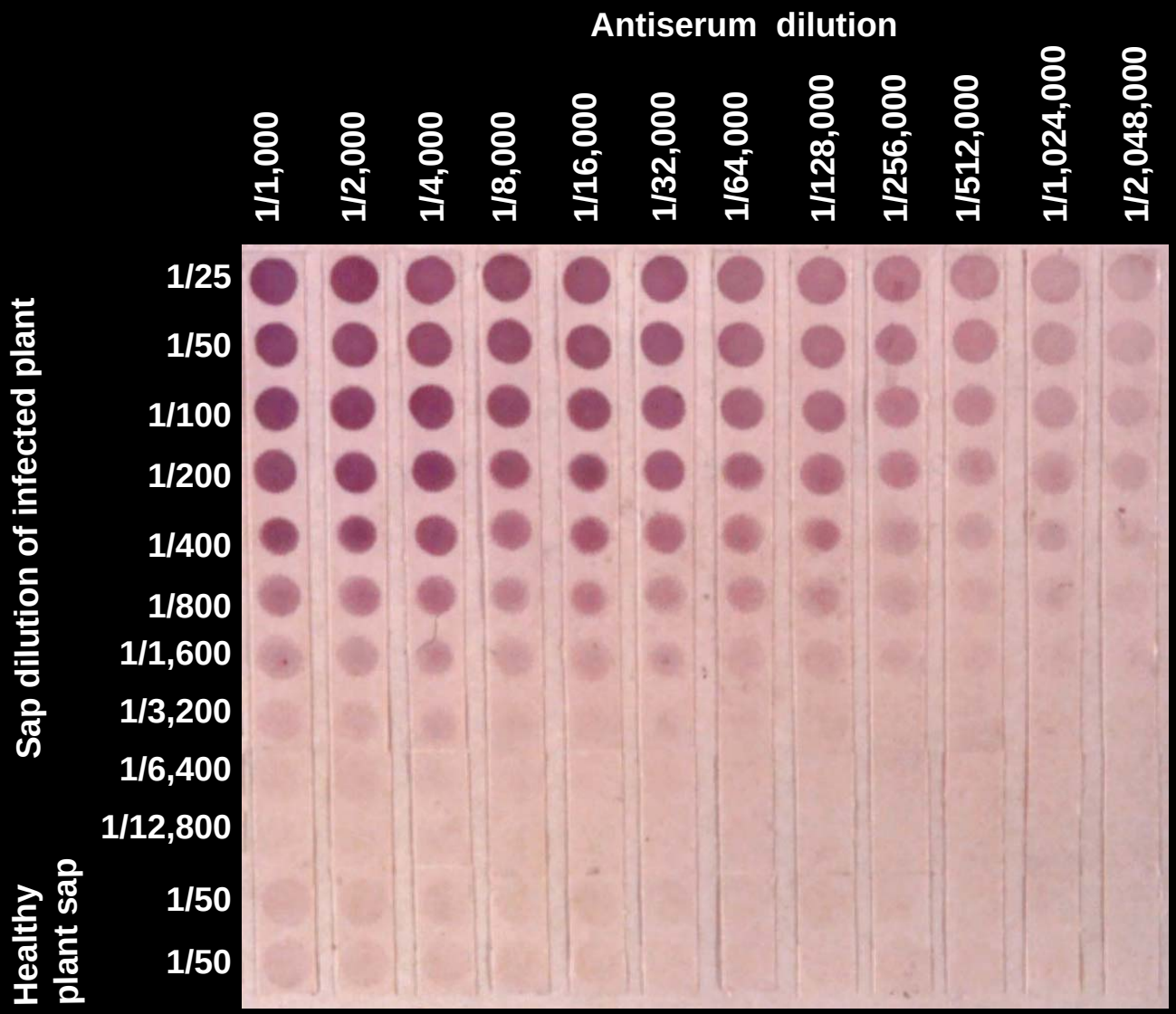
NCM-ELISA

stop

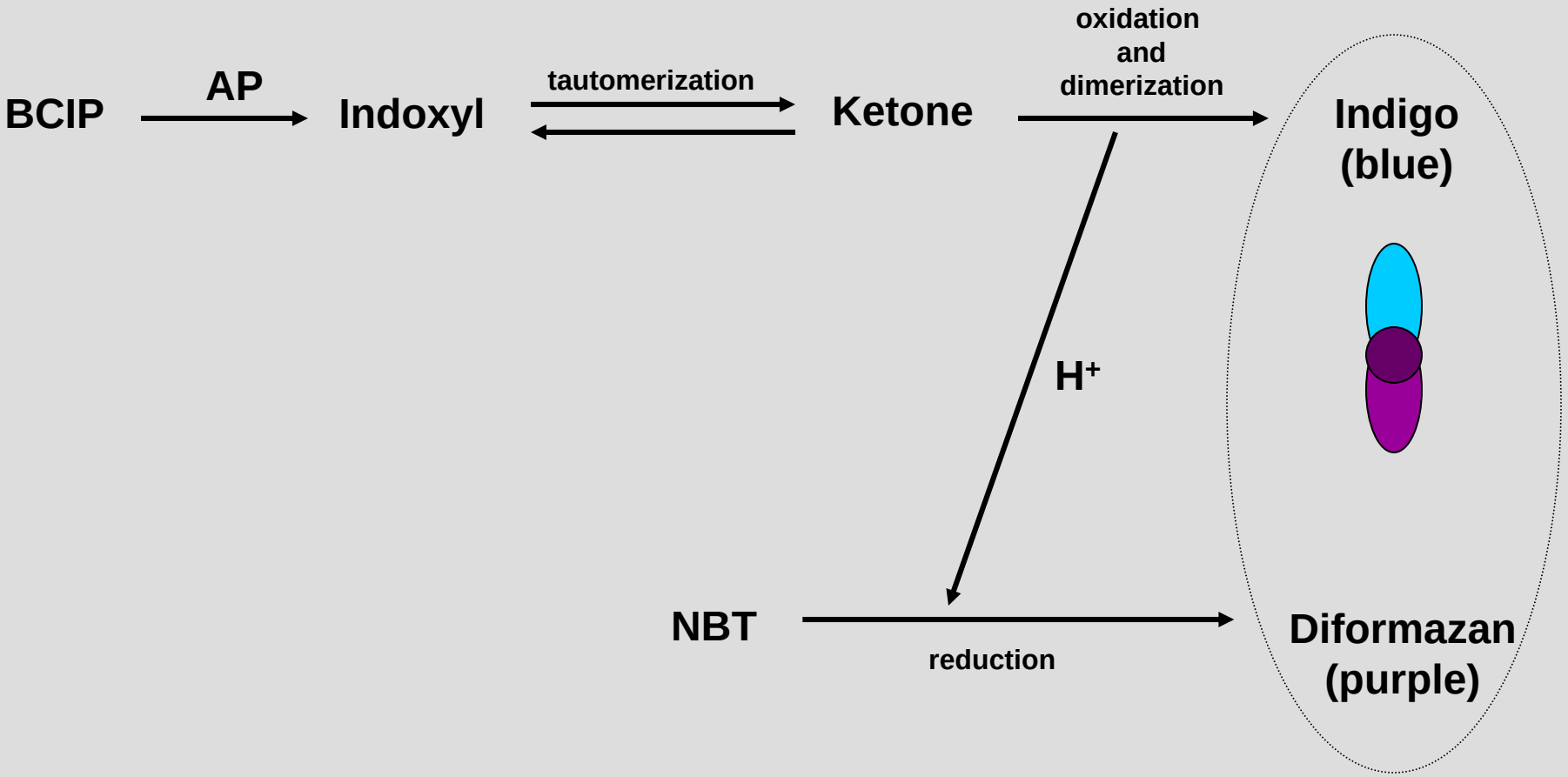


FUNDAMENTALS ON NCM - ELISA

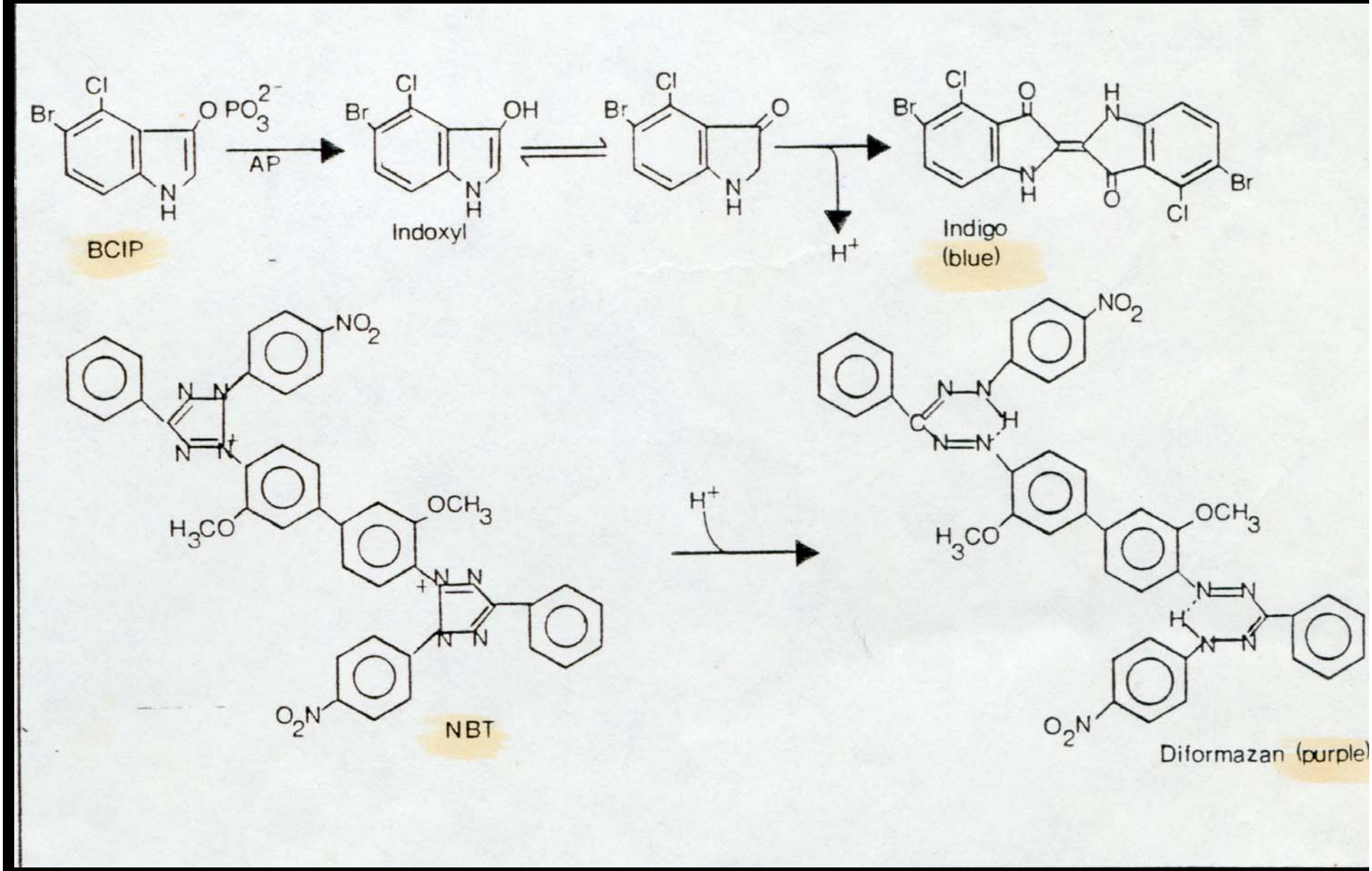
Antibody titration



Color development reaction catalyzed by allkaline phosphatase (AP) with BCIP as substrate combined with NBT

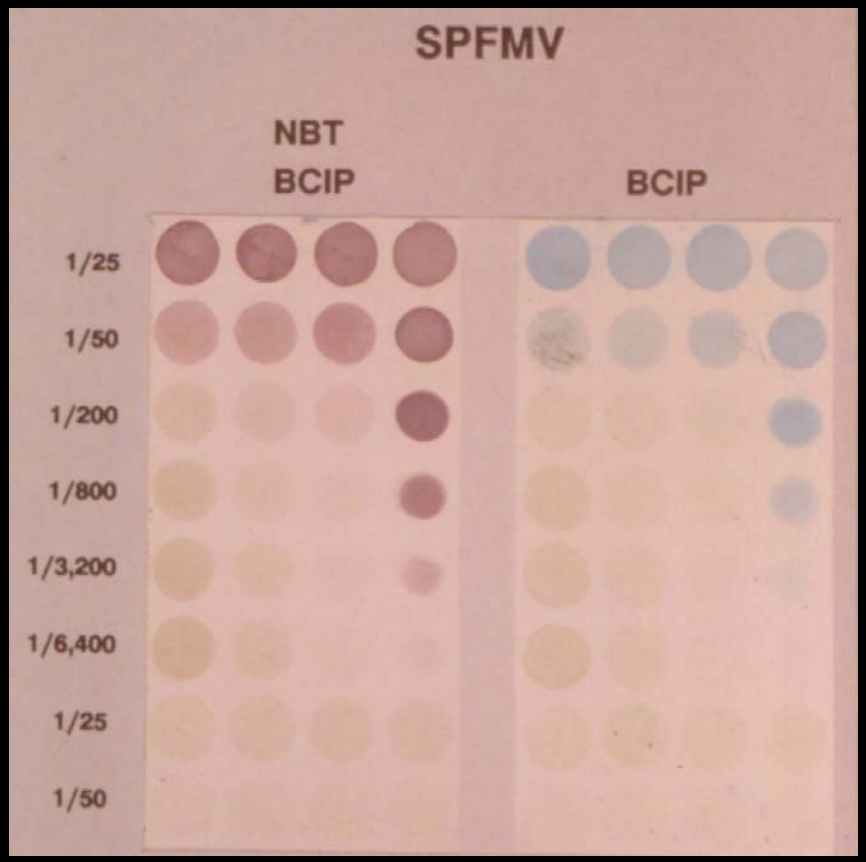


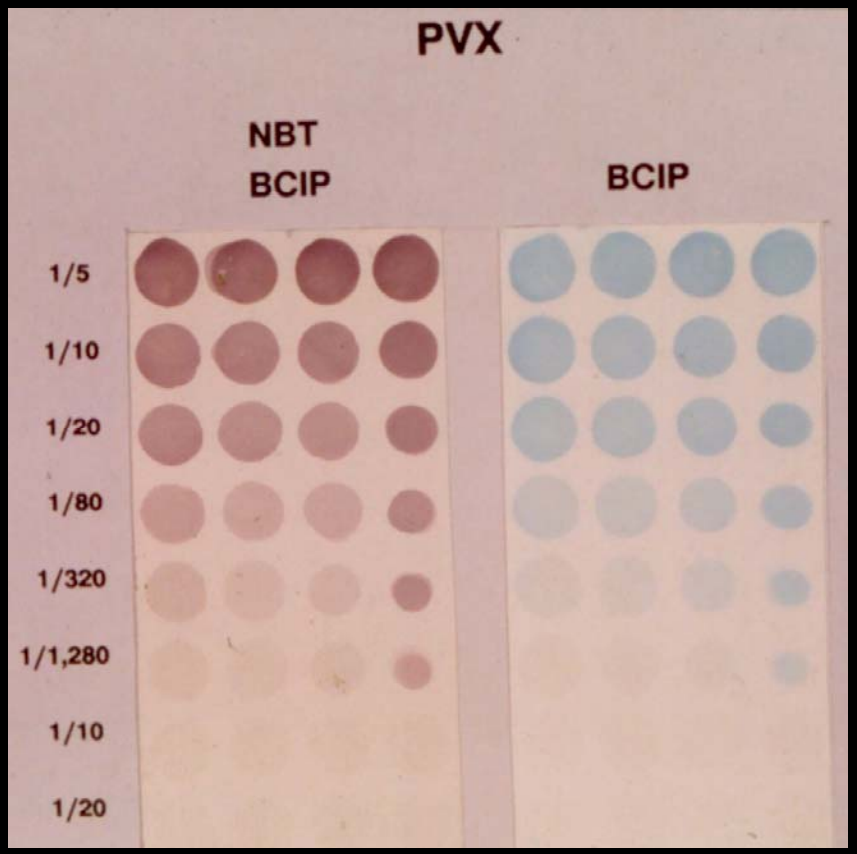
Color development reaction catalyzed by allkaline phosphatase (AP) with BCIP as substrate combined with NBT



Effect of substrate components concentration on the color development

Comparison of developed membranes





30 min | 2 to 3 h | 30 min | 2 to 3 h

Color development time