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The defectiveness of the subgroup F adenoviruses in vitro

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APPENDIX A: Abbreviations

A	ampere
AAV	adeno-associated virus
Ad	adenovirus
Ad-pol	adenovirus polymerase
Ala	alanine
AMV	avian myeloblastosis virus
ATP	adenosine triphosphate
BHK	baby hamster kidney
BMV	Brome mosaic virus
bp	base pair
BCIP	bromochloroindolyl phosphate
BRK	baby rat kidney
BSA	bovine serum albumin
°C	degrees celsius
C	cytosine
cAMP	cyclic adenosine monophosphate
cDNA	complementary deoxyribonucleic acid
CEF	chicken embryo fibroblasts
CPE	cytopathic effect
cpm	counts per minute
CTL	cytotoxic T lymphocyte
dATP	deoxy-adenosine triphosphate
DBP	DNA binding protein
dCMP	deoxy-cytidine monophosphate
dCTP	deoxy-cytidine triphosphate
DEAE-dextran	diethylaminoethlyl-dextran
DEP	diethyl pyrocarbonate
dGTP	deoxy-guanidine triphosphate

DIG	digoxigenin
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DMSO	dimethyl sulphoxide
DOTMA	N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammoniumchloride
dTTP	deoxy-thymidine triphosphate
EBV	Epstein-Barr virus
EDTA	ethylenediaminetetra-acetic acid
EM	electron microscopy
EMEM	Earle's minimal essential medium
EtBr	ethidium bromide
FCS	foetal calf serum
FFU	flourescent focus-forming unit
g	gravitational force
G	guanine
Gly	glycine
GMK	Green monkey kidney
HA	haemagglutination
H_20	water
HeBS	Hepes buffered saline
HEF	human embryo fibroblasts
HSV	herpes simplex virus
IFN	interferon
IL-6	interleukin-6
ITR	inverted terminal repitition
K/kDa	kiloDalton
kbp	kilobase pair
LA	Luria agar
LB	Luria broth
M	molar
mA	milliAmpere
MAb	monoclonal antibody

Met	methionine
mCi	milliCurie
MLP	major late promoter
mM	millimolar
m.o.i.	multiplicity of infection
mRNA	messenger RNA
m.u.	map unit
MW	molecular weight
NF	nuclear factor
nm	nanometre
NP40	nonidet P40
NTB	nitroblue tetrazolium salt
OD	optical density
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
PFU	plaque forming units
pg	picogram
p.i.	post-infection
pmol	picomole
pTP	precursor terminal protein
R	amino acid residues
R.I.	refractive index
R.I.P.A.	radioimmunoprecipitation assay
RNA	ribonucleic acid
RNase	ribonuclease
RNAsin	RNAse inhibitor
rpm	revolutions per minute
rRNA	ribosomal RNA
RT-PCR	reverse transcription - polymerase chain reaction

S	Svedberg unit
SDS	sodium dodecyl sulphate
s.i.	single infection
SV40	simian virus 40
TBS	Tris buffered saline
TCA	trichloroacetic acid
TEMED	N,N,N′,N′,-Tetramethylethylenediamine
TF	transcription factor
Tris	Tris-(hydroxymethyl)-aminoethane
Triton X100	polyethyleneglycol (9-10)-p-t-octylphenol
tRNA	transfer RNA
μCi	microCurie
μg	microgram
μl	microlitre
μm	micron
UV	ultraviolet
VA	virus-associated
Val	valine
VSV	vesicular stomatitis virus
WHO	World Health Organization
w.r.t.	with respect to

APPENDIX B: Compositions of buffers and media

1. Reagents for ELISA

Carbonate buffer pH 9.6

0.15M Na_2CO_3, 0.35M $NaHCO_3$. Make up before use in deionized distilled water.

Substrate solution

0.04% o-phenylamine diamine, 0.7% di-sodium tetraborate, 0.36% succinic acid, 0.08% urea hydroxide (all w/v).

Make up a fresh solution in deionized distilled water before use and keep in the dark until required.

3N HCl

Dilute fuming HCl 1:10 in deionized distilled water.

2. Preparation of glassware, plasticware and solutions for nucleic acid work

For work with DNA samples, sterilize all pipette tips, eppendorf micro-centrifuge tubes, SW40 polyallomer and SS34 centrifuge tubes by autoclaving.

Cleaning and acetylation of glass coverslips is carried out as follows: Dip coverslips 1M HCl, wash in distilled water, dip in 95% ethanol and wipe dry with gauze. Soak coverslips for 3 hours in Denhardt's medium (0.02% w/v BSA, 0.02% w/v polyvinylpyrrolidone, 0.02% w/v Ficoll, 3xSSC) at 65°C. Transfer to ethanol-acetic acid (3:1 v/v) for 20 minutes and allow to air dry. Acetylate as follows: Dip in 0.1M triethanolamine pH 8.0. Add acetic anhydride to 0.25% v/v and mix thoroughly for 10 minutes. Wash twice in water and once in 95% ethanol, air dry and store at room temperature until use.

For work with RNA samples siliconize glassware, plasticware and glass wool by soaking in 5% dichlorodimethylsilane in chloroform and then rinse several times with sterile water. Autoclave plasticware and glass wool, and bake glassware at 180°C for two hours before use. Soak eppendorf micro-centrifuge tubes and pipette tips in 0.1% v/v DEP in distilled water overnight, dry and autoclave to inactivate DEP.

Treat solutions required for RNA work with 0.1% v/v DEP and leave overnight. As Tris inactivates DEP, the solution without Tris must first be DEP-treated and autoclaved. The Tris solution must be filter sterilized and added to the solution after autoclaving. Heat SDS stock solutions to 60°C for two hours.

3. Spectrophotometric quantitation of nucleic acids

A reading at a wavelength of 260nm allows calculation of the concentration of nucleic acid in the sample. An OD of 1 corresponds to about 50μg/ml for double-stranded DNA, 40μg/ml for single-stranded DNA and RNA, and about 20μg/ml for single-stranded oligonucleotides. A ratio between the readings at 260nm and 280nm (OD_{260}/OD_{280}) gives an indication of the purity of the nucleic acid. OD_{260}/OD_{280} values of 1.8 and 2.0 are obtained for pure preparations of DNA and RNA respectively.

4. Antibiotics

Ampicillin

Stock solution: 25mg/ml of the sodium salt of ampicillin in water. Filter through a disposable 0.22μm filter and store in 1ml aliquots at -20°C.

Tetracycline

Stock solution: 12.5mg/ml tetracycline hydrochloride in ethanol/water (50% v/v). Store in 1ml aliquots at -20°C.

Kanamycin

Stock solution: 25mg/ml in water. Sterilize by filtration and store in 1ml aliquots at -20°C.

5. Reagents for bacterial cultivation and DNA extraction

Luria-Bertani broth (LB broth)

Tryptone	10g/l
Yeast extract	5g/l
NaCl	5g/l*
Glucose	1g/l*

*The NaCl concentration is half of that in the original recipe and glucose is additional. Dissolve in distilled water, adjust the pH to 7.5 with NaOH, and sterilize by autoclaving.

Luria-Bertani agar (LB agar)

Recipe as for LB broth with the addition of 15g/l of Bacto-agar.

STE

0.1M NaCl, 10mM Tris-Cl pH 7.4, 1mM EDTA

GET

50mM glucose, 25mM Tris-Cl pH 8.0, 10mM EDTA

Potassium acetate

Add 11.5ml glacial acetic acid and 28.5ml water to 60ml 5M potassium acetate. This solution is 3M w.r.t. potassium and 5M w.r.t. acetate.

6. Reagents for PAGE

Acrylamide stock solution

Acrylamide-bisacrylamide (30:0.8). Make up in distilled water, add 5g Amberlite MB1 to 100ml acrylamide solution and stir for 60 minutes. Pour through a scinterred glass funnel to remove the Amberlite resin. Store at 4°C in the dark.

Stacking gel buffer

0.5M Tris-Cl pH 6.8. Autoclave and store at 4°C.

Resolving gel buffer

1.5M Tris-Cl pH 8.8. Autoclave and store at 4°C.

10% SDS

10% ammonium persulphate

Make up fresh before use.

Reservoir buffer stock

0.25M Tris-Cl pH 8.3, 1.92M glycine, 1% w/v SDS. Store at 4°C.

5x Sample dissociation buffer

10% w/v SDS, 10% v/v 2-mercaptoethanol, 40% v/v glycerol, 0.02% w/v bromophenol blue in 1.25M Tris-Cl pH 6.8. Add a 1/5 volume of dissociation buffer to each sample and heat to 95°C for 10 minutes before loading into wells.

Recipe for 12% resolving gel (50ml)

water	16.75ml
1.5M Tris-Cl pH 8.8	12.5ml
10% w/v SDS	0.5ml
Acrylamide-bisacrylamide (30:0.8)	20.0ml
10% w/v ammonium persulphate	250μl
TEMED	25μl

Recipe for 3.9% stacking gel (10ml)

water	6.1ml
0.5M Tris-Cl pH 6.8	2.0ml
10% w/v SDS	50μl
Acrylamide-bisacrylamide (30:0.8)	1.3ml
10% w/v ammonium persulphate	50μl
TEMED	10μl

Gel fixative

40% v/v methanol, 7% v/v acetic acid in distilled water.

7. Reagents for agarose gel electrophoresis

TAE buffer (1x)

40mM Tris-Cl, pH 8.0, 20mM sodium acetate, 2.5mM EDTA.

TBE buffer (1x)

89mM Tris-borate, 89mM boric acid, 2mM EDTA, pH 8.0.

DNA sample loading buffer (10x)

Prepare a saturated sucrose solution containing 2% w/v SDS, 100mM EDTA, and 0.1% w/v bromophenol blue. Add a 1/10 volume of loading buffer to the DNA sample before loading into the wells of an agarose gel.

[illegible]nts for DNA transfections

2 x Hepes-buffered saline (HeBs)

NaCl	16.4 g/l
Hepes acid	11.9 g/l
Na_2HPO_4	0.21 g/l

Adjust pH to 7.05 with 5M NaOH and filter sterilize.

Tris-buffered saline (TBS)

Solution A		Solution B	
NaCl	80 g/l	$CaCl_2$	15g/l
KCl	3.8 g/l	$MgCl_2$	10 g/l
Na_2HPO_4	2 g/l		
Tris base	30 g/l		

Adjust pH to 7.5

Filter sterilize both solutions and store at -20°C.

For 100 ml, add 10 ml solution A to 89 ml water. While stirring rapidly, add 1 ml solution B slowly, drop by drop. Filter sterilize and store at 4°C. Mix well before use.

9. Stock solutions for hybridizations

20xSSC

Dissolve 175.3g of NaCl and 88.2g of citric acid in 800ml of distilled water. Adjust the pH to 7.0 using a 10N solution of NaOH. Adjust the volume to 1 litre, dispense into 200ml aliqouts and sterilize by autoclaving.

20xSSPE

Dissolve 174g of NaCl, 27.6g $NaH_2PO_4.H_2O$, and 7.4g of EDTA in 800ml of distil water. Adjust the pH to 7.4 with a 10N solution of NaOH. Adjust the volume to 1 litre, dispense into 200ml aliquots and sterilize by autoclaving.

50x Denhardt's solution

Ficoll	5g
polyvinylpyrrolidone	5g
BSA (pentax fraction V)	5g
H_2O	to 500ml

Filter through a 0.45μm disposable millipore filter. Aliqout in 30ml volumes and store at -20°C.

Deionized formamide

Mix 50ml formamide and 5g of Amberlite MB1 resin and stir for 30 minutes at room temperature. Filter twice through Whatman No. 1 filter paper to remove the resin. Store 1ml amd 10ml aliquots at -20°C.

APPENDIX C: List of suppliers

Item	Manufacturer
Plasticware	
6 well multidishes (9.6cm^2)	A/S Nunc, Denmark
24 well multidishes (1.9cm^2)	Nunc
50mm dishes	Sterilin Ltd, U.K.
90mm dishes	Sterilin
25cm^2 tissue culture flasks	Sterilin
75cm^2 tissue culture flasks	Sterilin
150cm^2 tissue culture flasks	Sterilin
72 well Nunc-Immuno plates	Sterilin
micro-centrifuge tubes, 1.5ml	Eppendorf GmbH, Germany
Falcon polypropylene tubes 15ml, 50ml	Becton Dickinson Inc. U.S.A.
Ultracentrifuge tubes	
Polyallomer centrifuge tubes	Beckman Instruments Inc. U.S.A.
Ultra clear™	Beckman Instruments
Sorvall SS34 tubes	Du Pont, de Nemours GmbH, Germany

Antibodies

Fluorescein isothyanate conjugated rabbit anti-mouse immunoglobulin	Dakopatts A/S, Denmark
Fluorescein isothyanate conjugated rabbit anti-horse IgG	Nordic Biochemicals BV, The Netherlands
Anti-digoxigenin-fluorescein Fab fragments	Boehringer Mannheim GmbH, Germany
Polyclonal antiserum to Ad5 hexon	Wellcome Diagnostics Ltd., U.K.
Peroxidase-conjugated rabbit immunoglobulins to mouse immunoglobulins	Dakopatts
Peroxidase-conjugated rabbit immunoglobulins to guinea pig immunoglobulins	Dakopatts

Antibiotics

Ampicillin, sodium salt	Sigma Chemical Company, U.S.A.
Tetracycline hydrochloride	Sigma
Kanamycin A	Sigma

Nucleic acids

Plasmids pBR322, pSPT18, pSPT19	Boehringer Mannheim
pMAM-neo	Clontech Inc., U.S.A.
Primers	Department of Biochemistry, UCT, RSA
Bacteriophage lambda DNA	Boehringer Mannheim
DNA from herring sperm	Boehringer Mannheim
yeast tRNA	Boehringer Mannheim

Radioisotopes

Phosphorus-32, carrier free, 370MBq/ml, 10mCi/ml	Amersham International plc, U.K.
Deoxycytidine 5′-[α-^{32}] triphosphate, triethylammonium salt, 370MBq/ml, 10mCi/ml (>400Ci/mmol)	Amersham International
L-[^{35}S]Methionine *in vivo* cell labelling grade, 370MBq/ml, 10mCi/ml (>1000Ci/mmol)	Amersham International
L-[^{35}S]Methionine, 555MBq/ml, 15mCi/ml (>1000Ci/mmol)	Amersham International
[methyl,1′,2′-^{3}H]Thymidine 37MBq/ml, 1mCi/ml (100-130Ci/mmol)	Amersham International
Rainbow™ protein molecular weight markers: ^{14}C-labelled mw range 14,300-200,000, 148kBq/ml, 4μCi/ml	Amersham international

Membranes and filter paper

Hybond™-C nitrocellulose membrane (pore size 0.45μm)	Amersham International
Hybond™-N nylon membrane (pore size 0.45 μm)	Amersham International
Hybond™-C gridded membranes	Amersham International
BA85 nitrocellulose membrane filters, 25mm (pore size 0.45μm)	Schleicher and Schuell GmbH, Germany
DE-81 membrane filters, 23mm	Whatman International, U.K.

Whatman no.1 filter paper	Whatman International

Photographic materials

Ilford FP4 medium speed (125ASA) black and white film, 35mm	Ciba Geigy (Pty) Ltd, Switzerland
Hyperfilm-MP	Amersham International
Hyperfilm-βmax	Amersham International
Ilford ID11 developer	Ciba Geigy
Ilford Ilfospeed developer	Ciba Geigy
Ilford Hypam fixer	Ciba Geigy
Adefo X-ray developer and fixer	Adefo chemicals GmbH, Germany

Chromatography

Sephadex G-50	Pharmacia Fine Chemicals AB, Sweden
Sephacryl S-1000	Pharmacia
Oligo(dT) cellulose	Boehringer Mannheim
Protein-A-Sepharose CL-4B	Sigma

Enzymes

Restriction endonucleases	Amersham International and Boehringer Mannheim
Lysozyme	Boehringer Mannheim
Pronase	Boehringer Mannheim
Proteinase K	Boehringer Mannheim
Alkaline phosphatase from calf intestine (CIP)	Boehringer Mannheim

DNA polymerase, T4	Boehringer Mannheim
DNA polymerase, Taq	New England Biolabs Inc, U.S.A.
Klenow enzyme, DNA polymerase I large fragment	Boehringer Mannheim
DNA ligase, T4	Boehringer Mannheim
Nuclease S1	Boehringer Mannheim
Avian myeloblastosis virus (AMV) reverse transcriptase	Boehringer Mannheim
RNase-free DNase	Boehringer Mannheim
DNase	Boehringer Mannheim
RNase	Boehringer Mannheim
*Bal*31	Boehringer Mannheim
cycloheximide	Boehringer Mannheim
trypsin	Sigma

<u>Miscellaneous reagents</u>

Mouse monoclonal antibody isotyping kit	Amersham International
Nick translation kit	Amersham International
DIG nucleic acid detection kit	Amersham International
Geneclean™ kit	Bio 101 Inc., U.S.A.
Rabbit reticulocyte lysate	Amersham International
Human placental ribonuclease inhibitor (RNasin)	Boehringer Mannheim
Dithiothreitol (DTT)	Boehringer Mannheim
Deoxyribonucleotide triphosphates (dNTPs)	Boehringer Mannheim
Bovine serum albumin (BSA)	Boehringer Mannheim

Blocking reagent for nucleic acid hybridizations	Boehringer Mannheim
spermidine	Boehringer Mannheim
'Amberlite' monobed resin MB-1	BDH Chemicals Ltd., U.K.

Organic chemicals

Acetone	Merck GmbH, Germany
Acetic acid (Glacial)	Merck
Acetic anhydride	Merck
2-butanol	Merck
Chloroform	Merck
Dichlordimethylsilane	Merck
Ethanol	Merck
Formaldehyde	Merck
Formamide	Merck
Freon 113	Merck
Hydrochloric acid	Merck
Isoamylalcohol	Merck
2-Mercaptoethanol	BDH
Isopropanol	Merck
Methanol	Merck
Phenol	Merck
Piperidine	BDH
Polyethylene glycol 1000	Merck
Polyethylene glycol 6000	BDH
1,1,2-Trichloro-Trifluoroethane	Sigma
Triethanolamine	Merck

Reagents for electrophoresis

Agarose	Bio-Rad Inc., U.S.A.
Nu-Sieve^{R} agarose	FMC Bioproducts Inc., U.S.A.
SeaKem^{R} GTG agarose	FMC Bioproducts
Acrylamide	Bio-Rad
Bis-acrylamide	Bio-Rad
TEMED	Bio-Rad
Ammonium persulphate	Bio-Rad

General chemicals

Ammonium acetate	Merck GmbH, Germany
Bacto-agar	Difco Laboratories Inc., U.S.A.
Boric acid	Merck
Calcium chloride	Sigma
Cesium chloride	Boehringer Mannheim
Citric acid, trisodium salt	Sigma
EDTA (Ethylenediaminetetra-acetic acid)	BDH
Ethidium bromide	Boehringer Mannheim
Formamide, GR	Merck
Ficoll, type 400-DL	Sigma
Glycine, GR	Merck
x-D(+)Glucose	Sigma
HEPES (2-[-4-(2-hydroxyethyl) -1-piperazinyl]-ethanesulfonic acid	Merck
N-Lauroylsarcosine, free acid	Sigma
Magnesium chloride, GR	Merck

Magnesium Sulphate, GR	Merck
Paraffin liquid	Tedro products, South Africa
Potassium chloride, Analar	BDH
Polyvinylpyrrolidone PVP-40, pharmaceutical grade	Sigma
di-Sodium hydrogen orthophosphate	BDH
di-Sodium tetraborate, Analar	BDH
Sodium acetate anhydrous, GR	Merck
SDS (n-Dodecylsulfate sodium salt)	Merck
Sodium hydroxide pellets, Analar	BDH
Sodium hydrogen carbonate, Analar	BDH
Sodium carbonate, Analar	BDH
Succinic acid, Analar	BDH
Sucrose, grade 1	Sigma
Tris(hydroxymethyl)aminomethane	Boehringer Mannheim
Tris(hydroxymethyl)aminomethane hydrochloride	Boehringer Mannheim
Tween^{R} 20 (Polyoxyethylene sorbitan monolaurate)	Merck
Tryptone	Oxoid Ltd., U.K.
Tablets of Urea, hydrogen peroxide	BDH
Yeast extract	Sigma

All reagents used were of the highest quality available.

APPENDIX D: Statistical evaluation of results

1. Wilcoxon's signed rank sum test

The Wilcoxon's signed rank sum test is a distribution-free or non-parametric statistical test i.e. no assumption is made about the distribution as in the student's t-test. The hypotheses to be tested usually relate to the nature of the distribution as a whole rather than to the values assumed by some of its parameters, hence the term non-parametric to describe the test. If the population distributions are normal with the same variance, the t-test is the most efficient test, but the rank test (Wilcoxon, Mann-Whitney, or Kendall) has a relative efficiency of 0.96. If the distributions, however, are not normal, the relative efficiency of the rank test is never less than 0.86 and may be infinitely high. For detecting a shift in location, therefore, the rank test is never much worse than the t-test, and can be very much better.

This test was chosen as the sample sizes were small and there may be possible non-normality.

One-sample test for location

Null hypothesis: the distribution of a random variable x is symmetric about zero. The Wilcoxon's signed rank sum test works as follows: The observations are put is ascending order of magnitude, ignoring the sign, and given the ranks 1 to n' (zero values being ignored). Let T_+ be the sum of the ranks of the positive values and T_- that of the negative. On the null hypothesis T_+ and T_- would not be expected to differ greatly; their sum $T_+ + T_-$ is $\frac{1}{2}n'(n'+1)$, so an appropriate test would consist in

evaluating the probability of a value of, say, T_+ equal to or more extreme than that observed. For large values of n', T_+ and T_- are approximately normally distributed with variance $n'(n'+1)(2n'+1)/24$, and a standardized normal deviate, with continuity correction, is given by

$$\frac{-|T+-\frac{1}{4}n'(n'+1)|-\frac{1}{2}}{\sqrt{\{n'(n'+1)(2n'+1)/24\}}}$$

Tied ranks equal to the mean are given to numerically equal observations. This feature reduces the variance of T_+ by $(t_3-t)/48$ for each group of tied ranks.

Data was stored in Edlin files and the relevant 2 files containing 4 items (4 replicates) each compared using a computer software statistics package TADPOLE version III. Ten observations (all possible combinations of differences between 2 groups of 4 values each) for each comparison were signed and ranked by the Wilcoxon's signed-ranks matched-pairs test and the significance determined at the 5% level ($P<0.05$).

2. Linear regression analysis

A computer programme was compiled by Z.Berkowitz to plot linear regression slopes and to determine the statistical variance between slopes. For equations see Armitage (1977). The explanation below applies to the use of 4 replicates/y values for each x dose.

Analysis of variance for each regression slope

A variance ratio (VR) was determined and compared with the f distribution on 2 and 12 degrees of freedom. The statistic F is a variance ratio that handles large variations

of means from the regression line, and this value was compared with the *f* distribution at 1 and 2 degrees of freedom.

A statistic *G* was calculated which compares variance between two slopes. This statistic does not assume a common residual variance. This value compared with a chi-square value at 1 degree of freedom indicates significance (α=0.01).

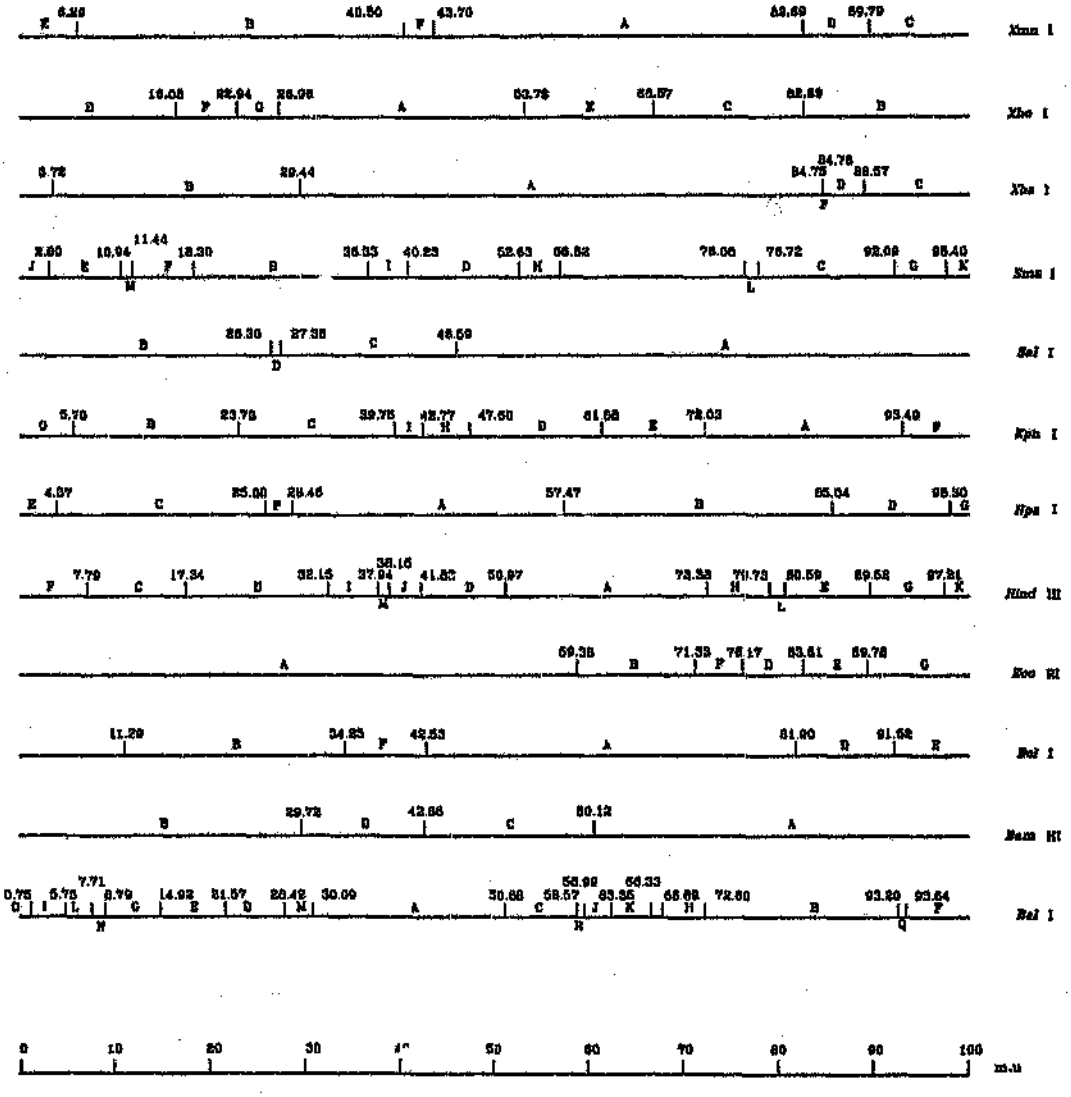
Analysis of variance between regression slopes assuming a common residual variance

The VR for the sum of squares between slopes was compared with the *f* distribution at 1 and 28 degrees of freedom. The statistic *T* provides a comparison of two slopes. Significance is determined by comparison with the *t* distribution at 28 degrees of freedom (α=0.01).

APPENDIX E: Restriction enzyme maps

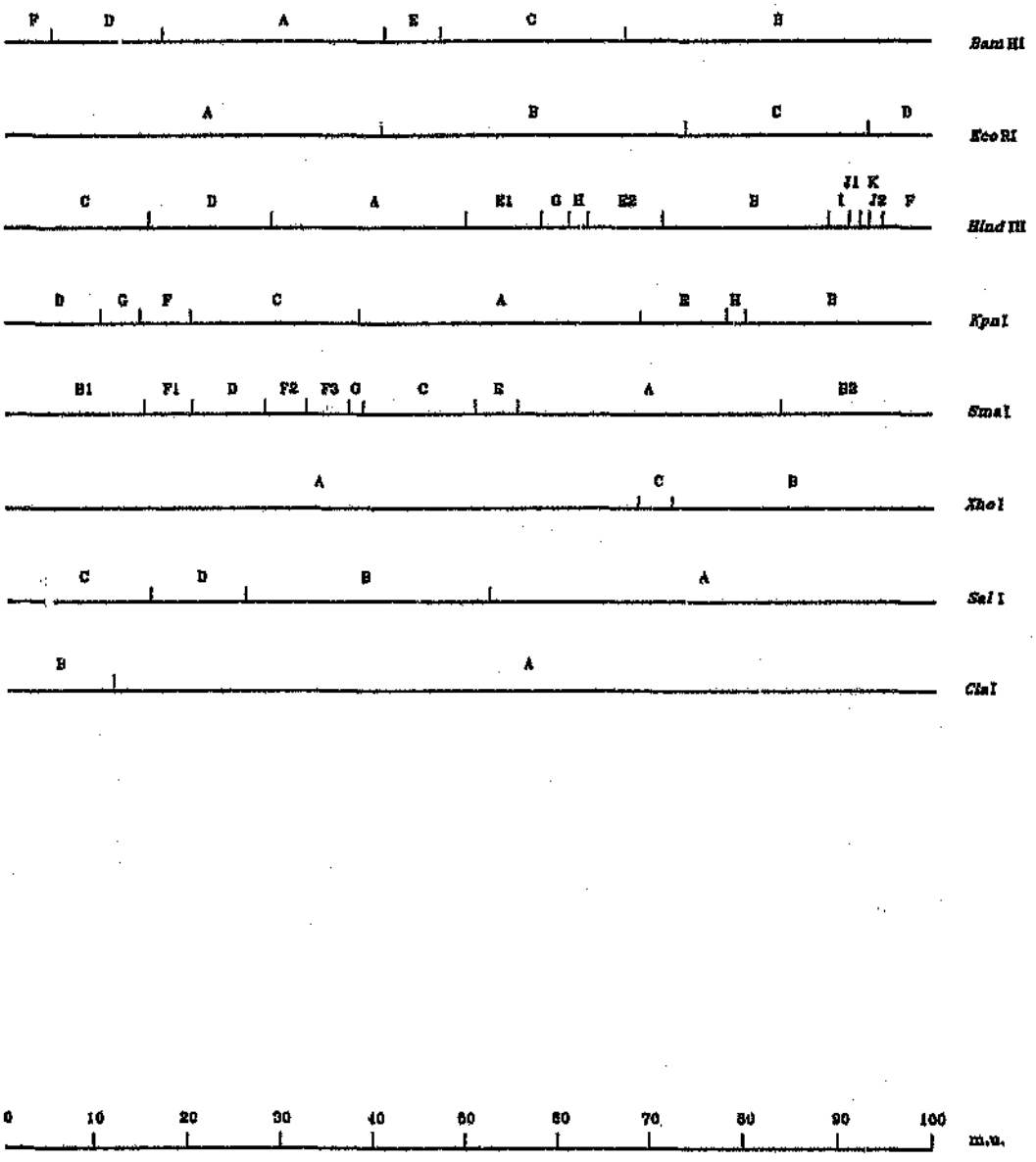
1. Adenovirus 2

(From Broker *et al*, 1984)



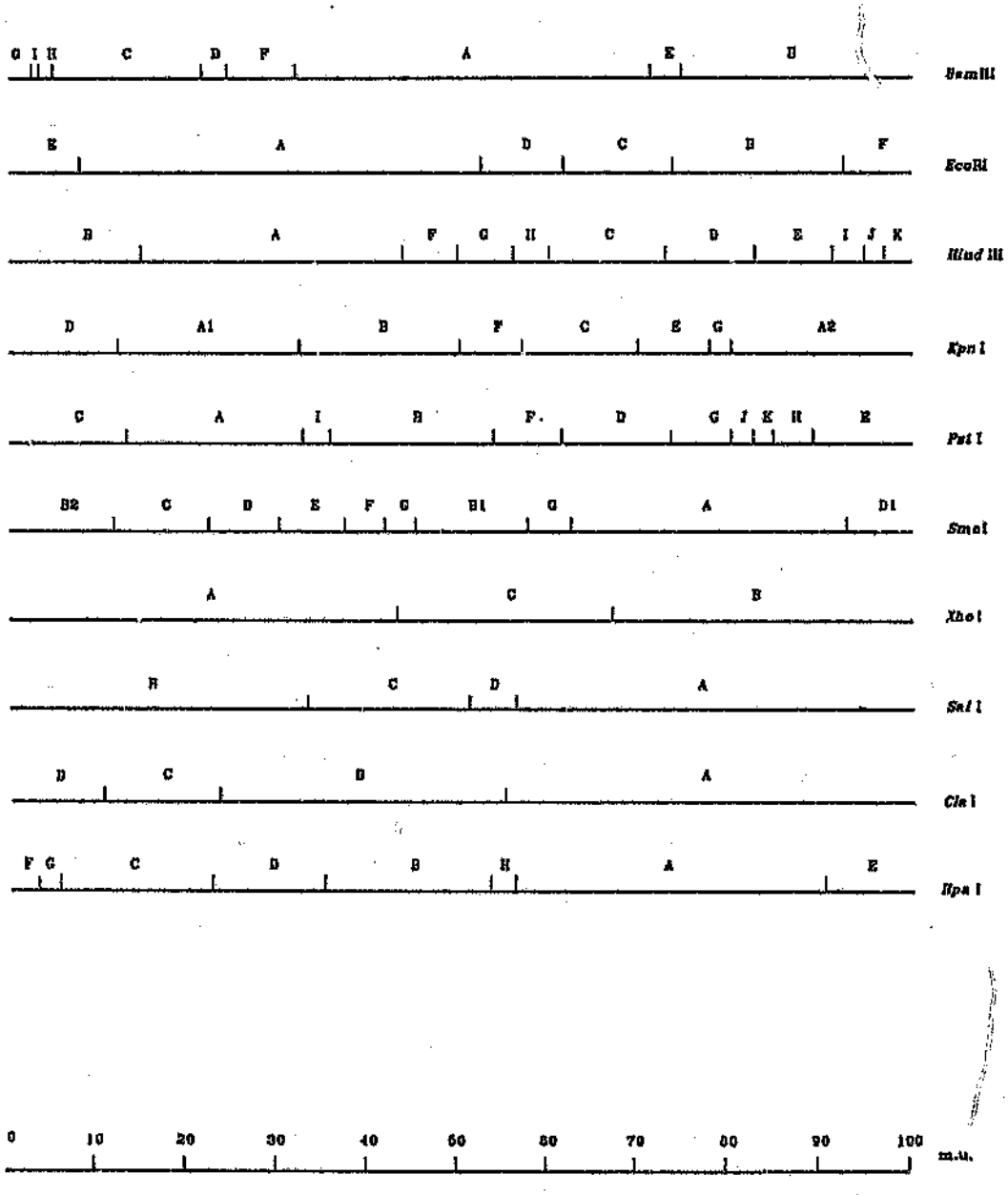
2. Adenovirus 40 genome type D1 (strain Hovi-X)

(From van der Avoort *et al*, 1989; *Sal*I and *Cla*I maps from van Loon *et al*, 1985b (genome type D4 strain Dugan))



3. Adenovirus 41 genome type D2 (strains N597 and 26341-77)

(From van der Avoort *et al*, 1989; *Sal*I and *Hpa*I maps from Allard *et al*, 1985 (strain D389); *Cla*I map from van Loon *et al*, 1985b (genome type D1 strain Tak))



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