

SELECTION OF DIAGNOSTIC ASSAYS FOR BVD CONTROL PROGRAMMES

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HOW TO DIAGNOSE BVD ?

- ◆ clinical signs - suggestive but *not conclusive*
- ◆ laboratory tests *only* reliable method
 - ◆ but which tests to ask for?
 - ◆ what are the differences between "similar" tests?
 - ◆ what are the disadvantages of each tests?
 - ◆ which tests complement each other best?
 - ◆ what samples to take?
 - ◆ herd or individual level?

PURPOSE OF DIAGNOSTIC INVESTIGATIONS

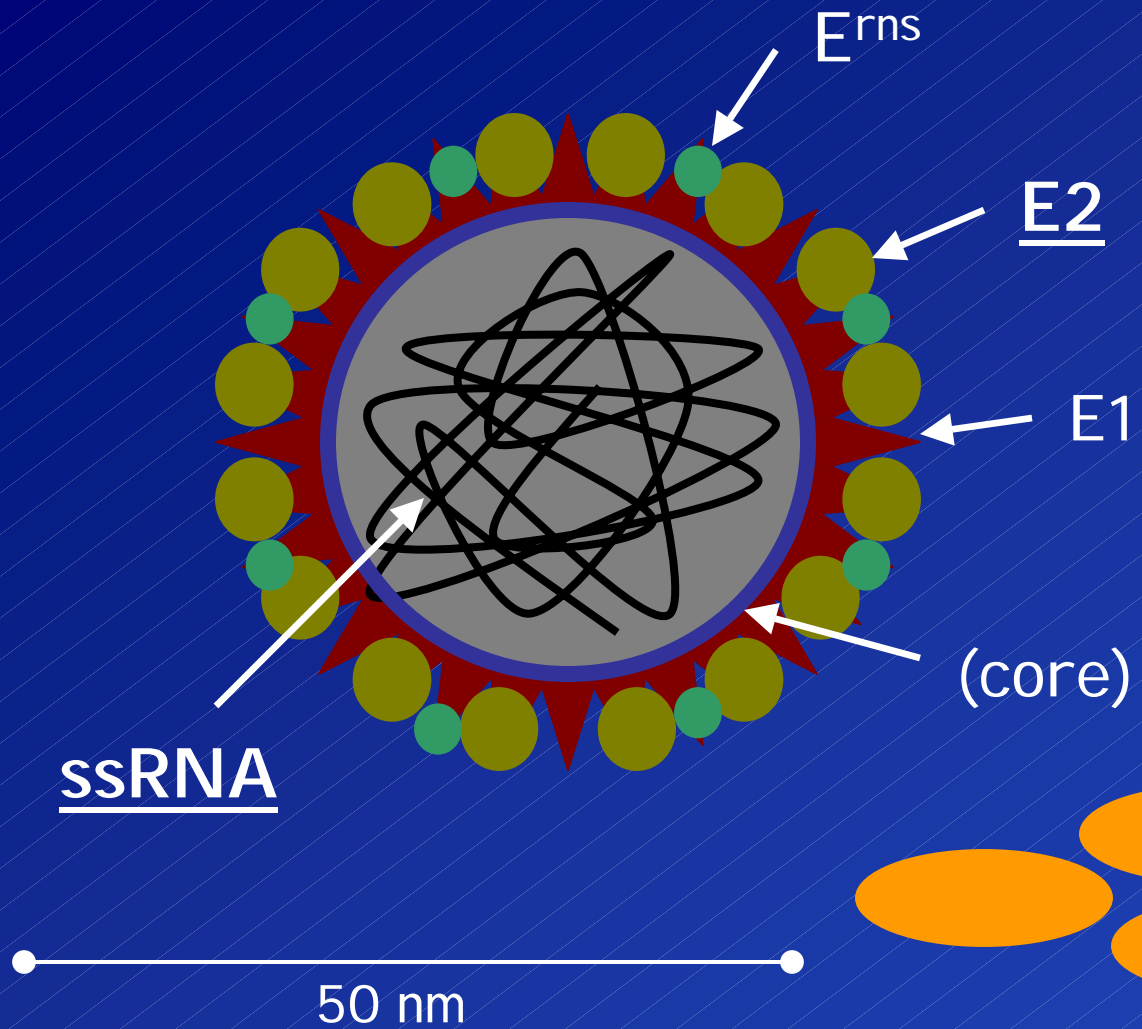
- ◆ *surveillance*
 - ◆ descriptive epidemiology
 - ◆ monitoring effect of control activities
- ◆ *herd level or individual animal testing*
 - ◆ determining immune status
 - ◆ identification of viremic animals
 - ◆ certification for trade or transport
 - ◆ individual case diagnostics
- ◆ *quality control*
 - ◆ documenting freedom of BVDV in e.g. semen / embryos
 - ◆ back up tests for individual use
 - ◆ calibration of assays

WHAT CAN BE DETECTED?

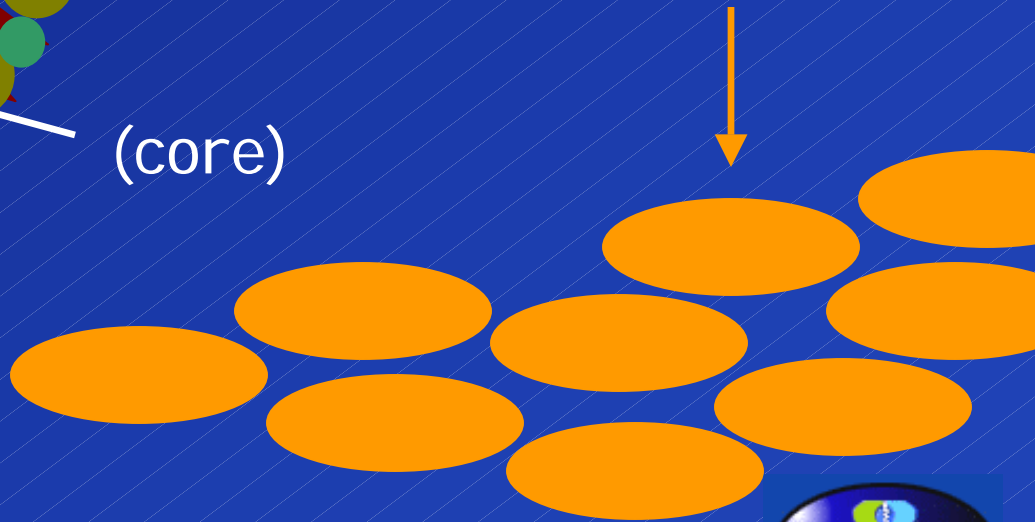
- ◆ *immunity to BVDV*
 - ◆ humoral / cellular
 - ◆ active / passive
 - ◆ susceptibility / immunotolerance
 - ◆ vaccination - natural infection
- ◆ *the virus*
 - ◆ infectious virus
 - ◆ viral antigens - NS3, Erns, E2, or combined
 - ◆ viral nucleic acid (RNA)
- ◆ *the virus strain* (species/subtype)

THE VIRUS

.. components of importance from a diagnostic point of view

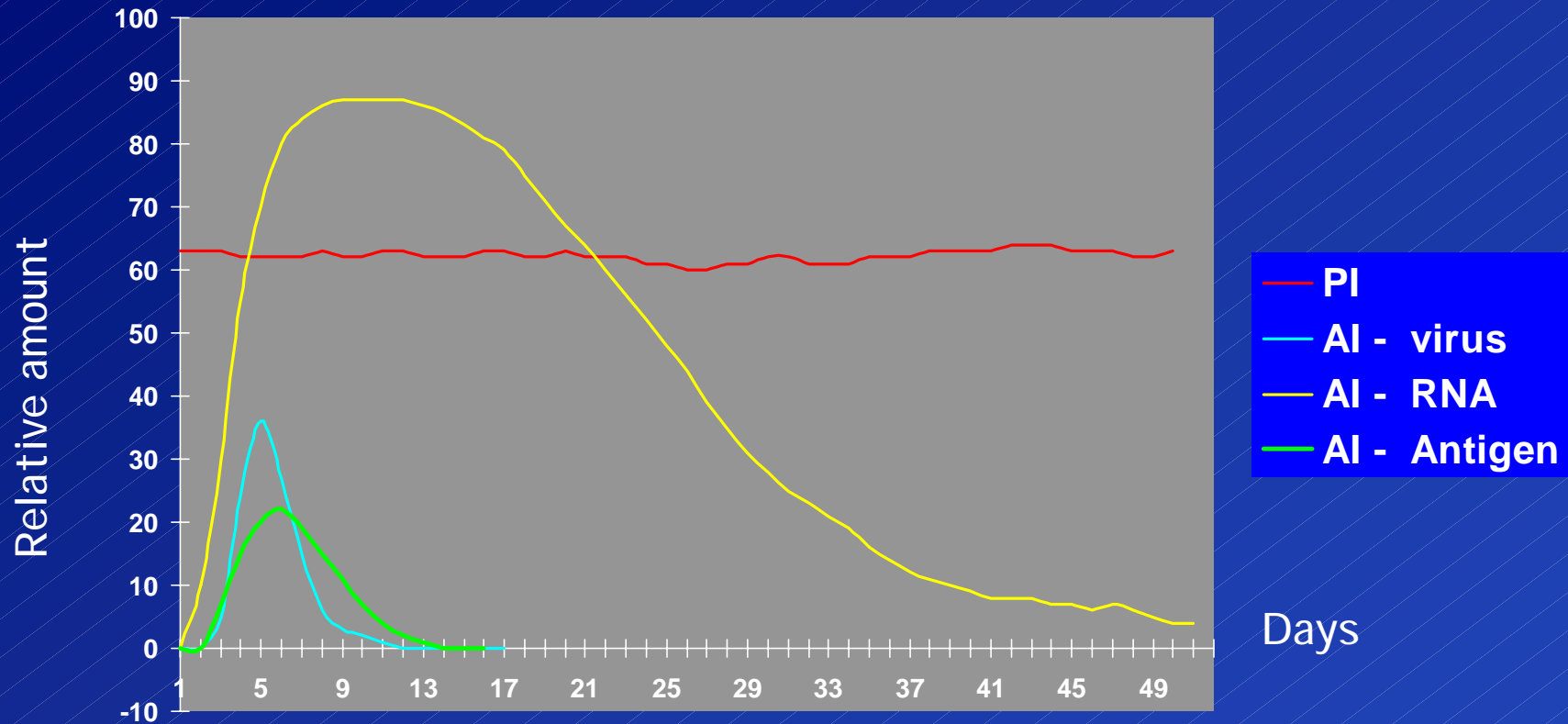


NS2-3
(p80/p125)



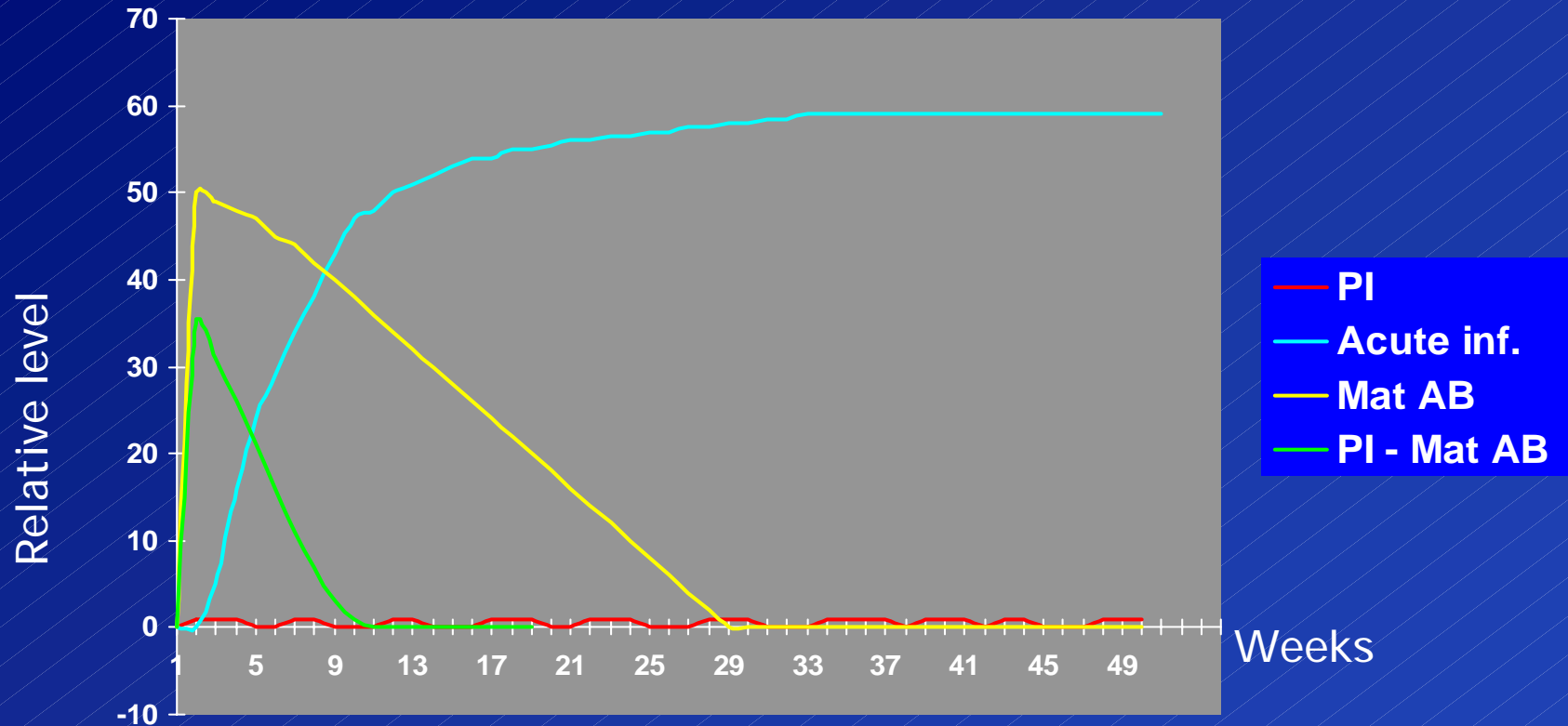
DETECTION OF VIRAL COMPONENTS BY TIME

- ◆ persistent (PI) vs acute infection (AI) - blood



DETECTION OF ANTIBODIES BY TIME

- ◆ acute vs persistent infection (PI) - serum



WHAT CAN BE *DEDUCED* FROM LAB RESULTS?

a) *Individual animals* :

- ◆ *BVDV immunity* (serology)
 - ◆ timing of infection - paired samples
 - ◆ nature of immune status - passive / active
 - ◆ antigenic properties of the infecting virus
- ◆ *virology*
 - ◆ acute vs persistent infection

WHAT CAN BE *DEDUCED* FROM LAB RESULTS?

b) Herd level :

- ◆ *BVDV immunity* (serology)
 - ◆ BTM / spot samples - active or recent infection
 - ◆ natural decline of antibody level
- ◆ *virology*
 - ◆ BTM RT-PCR - viremic cows
- ◆ *the virus strain*
 - ◆ reinfection with a new strain ?
- ◆ *over-all combined test results:*
 - ◆ does it make sense?

DESIRED PROPERTIES OF LAB TESTS

- ◆ sensitive & specific
- ◆ precise and accurate
- ◆ compatible with different sample materials
- ◆ well documented
- ◆ easy to perform, user “friendly”
- ◆ rapid
- ◆ cheap
- ◆ easily available
- ◆ suitable for automation

TESTS FOR DETECTION OF IMMUNITY

- ♦ *serological tests*
 - ♦ neutralisation tests - SNT / VNT
 - ♦ enzyme-linked immuno-sorbent assays - ELISA
 - ♦ indirect immunofluorescence / peroxidase staining
 - ♦ complement fixation
 - ♦ agar immunodiffusion
 - ♦ western blotting
 - ♦ *tests for cell-mediated immunity*
 - ♦ lymphocyte proliferation assay
- reference method*
- mass testing*

VIRUS NEUTRALISATION TEST - REFERENCE

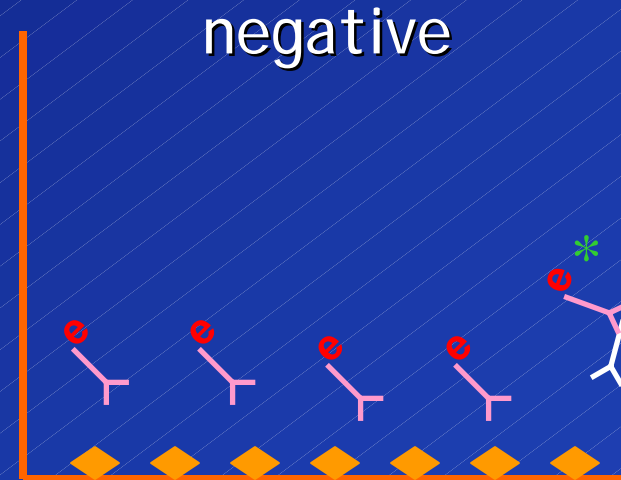
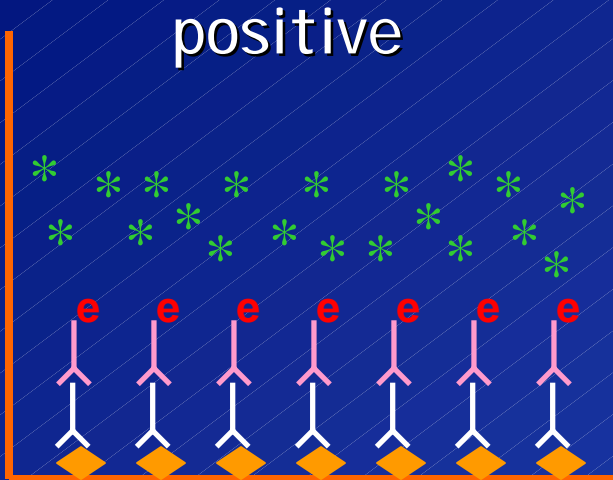
- ◆ *VNT/SNT/VN*
 - ◆ cell culture based
 - ◆ quantitates inhibitory effect on virus replication
 - ◆ detects anti-E2 antibodies
- ◆ *antigenic specificity*
 - ◆ variable titre against different virus strains
 - ◆ choice of challenge virus (cp) important
- ◆ *performance qualities*
 - ◆ specific and sensitive
 - ◆ resource demanding
 - ◆ not suitable for mass screening

ANTIBODY ELISAs

- ◆ Enzyme-Linked Immuno-Sorbent Assay
- ◆ common components:
 - ◆ antigen
 - ◆ enzyme-labelled reporter antibody
 - ◆ solid capture / liquid selection phases
 - ◆ chromogenic substrate
 - ◆ reference antisera (pos & neg controls)
- ◆ uses monoclonal ab's or specific antisera
- ◆ many different layouts . . .

ANTIBODY ELISAs - LAYOUT EXAMPLES

Indirect (amplifying)



—Y— serum ab

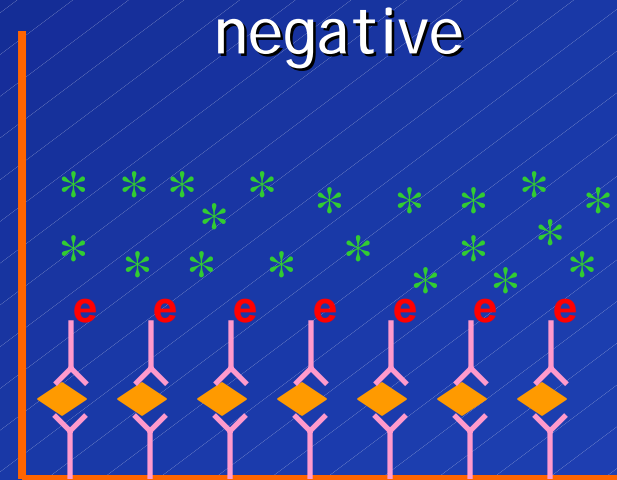
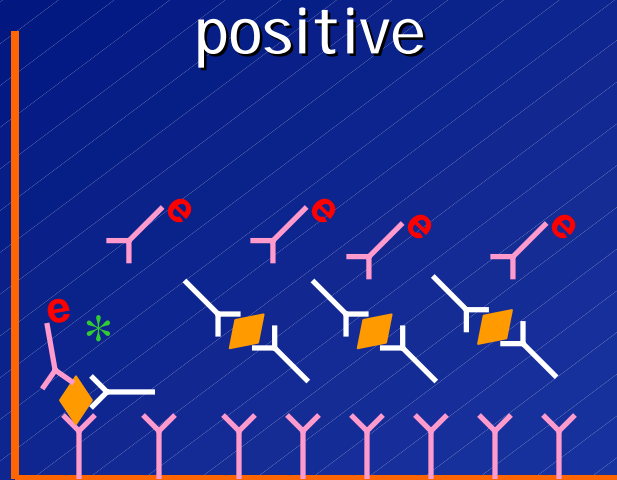
* substrate

◆ antigen

e—Y— enzyme labeled *anti-species* ab

ANTIBODY ELISAs - LAYOUT EXAMPLES

Blocking (competitive) (subtractive)



serum ab



antigen



substrate



assay ab



enzyme labeled ab

WHY USE ELISAs FOR BVD SEROLOGY ?

- ◆ *convenient:*

- ◆ independent of cell cultures
- ◆ easy to perform
- ◆ rapid
- ◆ cheap
- ◆ OK for different sample materials
- ◆ semi-quantitative
- ◆ suitable for automation
- ◆ availability

- ◆ *can be tailored for a specific task*

- ◆ choice of virus strain as antigen
- ◆ individual viral proteins as antigens
- ◆ potential to discriminate vaccination - natural infection

- ◆ *sensitivity & specificity varies from test to test !*

ANTIBODY ELISAs - OUTPUT FORMAT

- ◆ no titration = at best semi-quantitative
- ◆ how are cut-off values determined?
- ◆ inconclusive zone?
- ◆ *indirect assays*
 - ◆ "OD" = optical density values
 - ◆ absolute or relative readings
- ◆ *blocking assays*
 - ◆ percentage inhibition

WHICH ANTIBODY ELISA TO CHOOSE ?

- ◆ *in-house or commercial ?*
- ◆ *ask for detailed specifications*
 - ◆ nature of antigen - virus strain / protein(s)
 - ◆ antibodies - MABs /antisera
 - ◆ validation - published data ?
 - ◆ version history ?
 - ◆ solid-phase format ?
 - ◆ convenient in use ?

WEAK AND STRONG SIDES

- ◆ *antigen* :
 - ◆ purification procedure - structural vs NS proteins
 - ◆ glycoproteins - cover both BVDV-1 and -2 ?
- ◆ *antibodies*:
 - ◆ monoclonals ?
 - ◆ antisera ?
- ◆ *signal to noise ratio*:
 - ◆ sufficient to support semi-quantitative claims ?

TESTS FOR DETECTION OF BVDV

- ◆ *infectious virus*

- ◆ virus isolation in cell cultures
- ◆ animal inoculation

reference method

- ◆ *viral components*

- ◆ antigenic proteins
 - ◆ ag-ELISA
 - ◆ immuno-fluorescence & -histochemistry
 - ◆ flow cytometry
- ◆ ribonucleic acid
 - ◆ RT-PCR
 - ◆ NASBA, ligase chain reaction
 - ◆ hybridisation

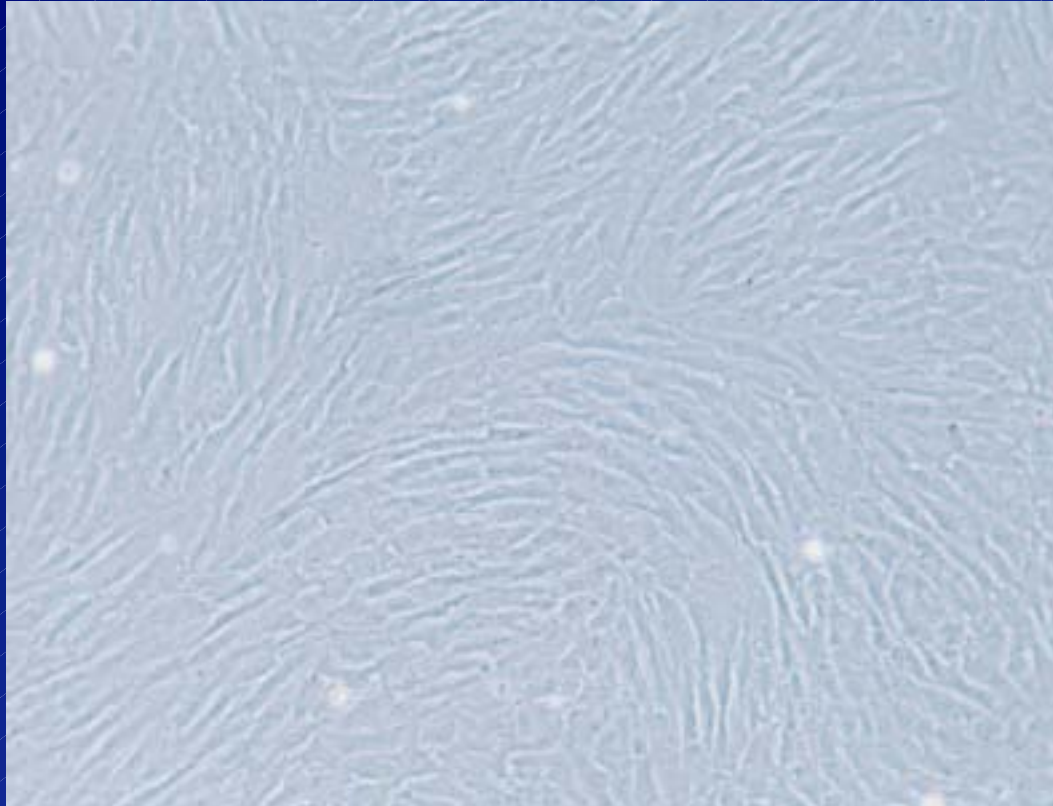
IPX

mass testing

CELL CULTURE VIRUS ISOLATION - REFERENCE

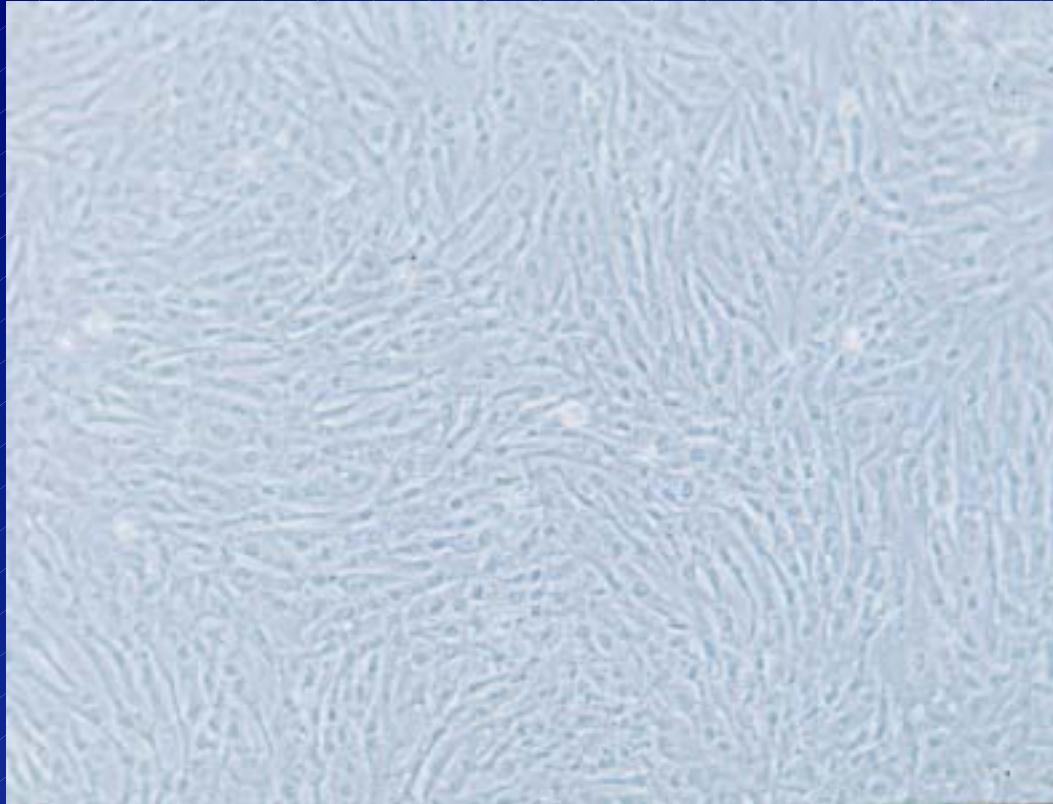
- ◆ *many different formats in use*
 - ◆ roller bottle cultures - serial passage
 - ◆ single passage microplate immunoperoxidase (IPX)
- ◆ *resource demanding*
 - ◆ requires well equipped laboratory
 - ◆ cell culture system / medium important
 - ◆ experienced staff important
- ◆ *important back-up method*
 - ◆ verification of results
 - ◆ obtaining and characterisation of viruses
 - ◆ support for research
 - ◆ biotype determination of virus isolates

BVDV CYTOPATHOGENICITY - 1



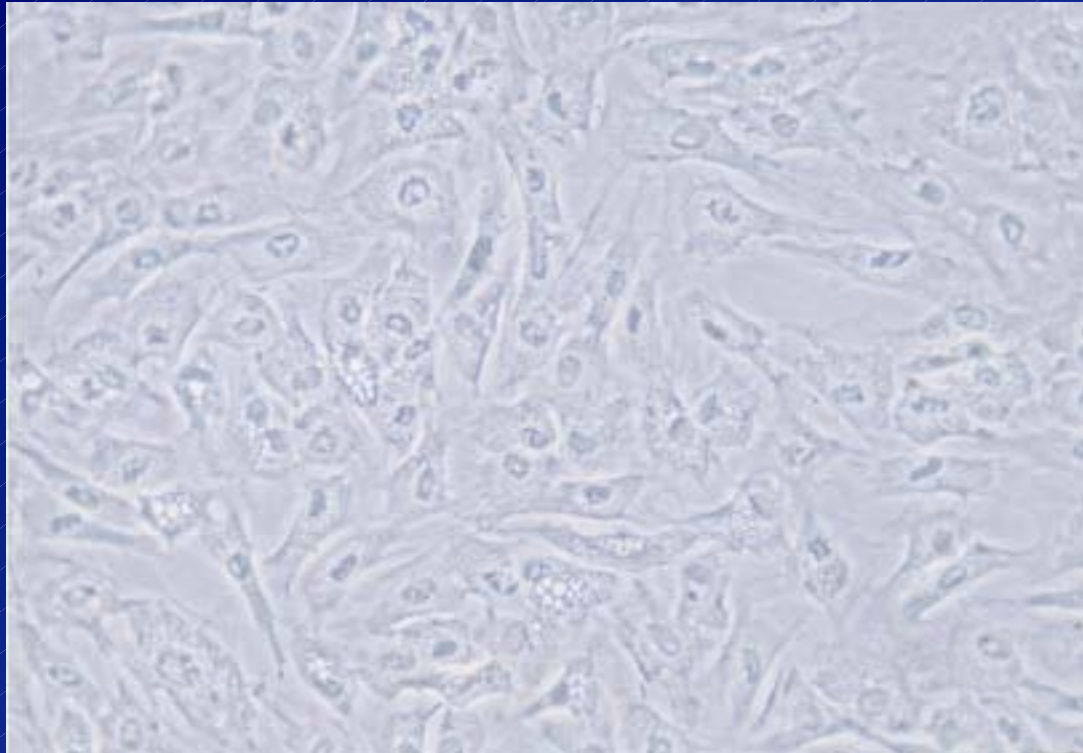
bovine
turbinate cells
-
uninfected
controls
-
normal cells

BVDV CYTOPATHOGENICITY - 2



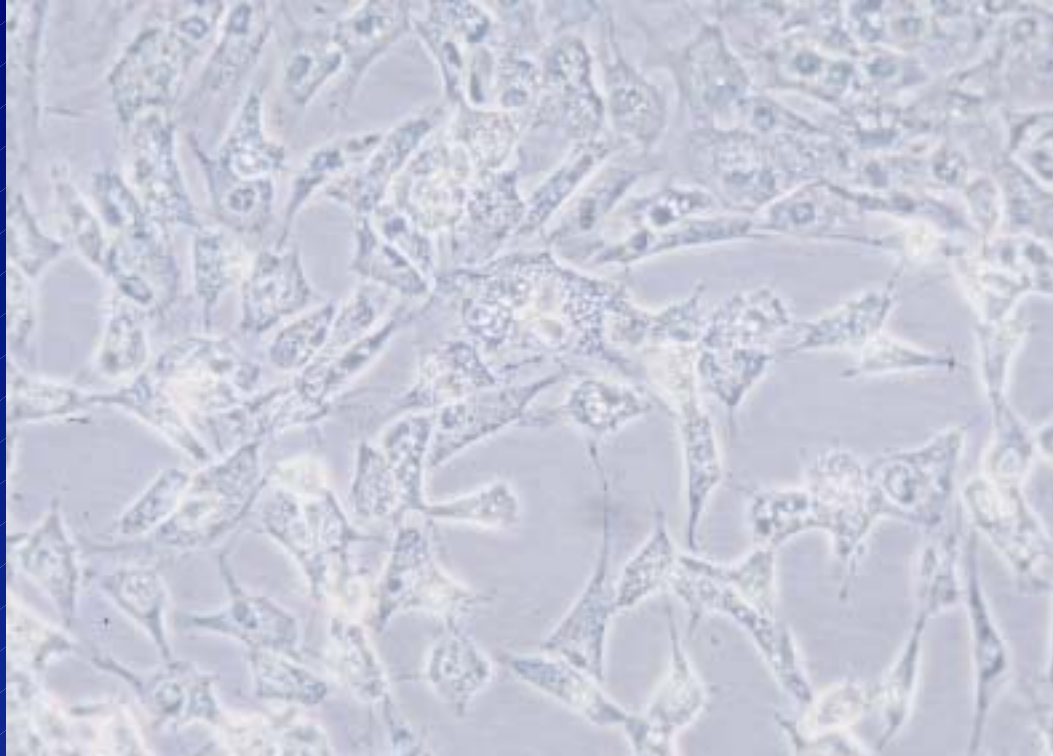
- bovine
- turbinate cells
-
- 24 hours after
- infection with
- cytopathogenic
- (cp) BVDV
-
- pyknotic nuclei

BVDV CYTOPATHOGENICITY - 3



bovine
turbinate cells
-
2 days post
infection with
cp BVDV
-
marked
apoptosis

BVDV CYTOPATHOGENICITY - 4

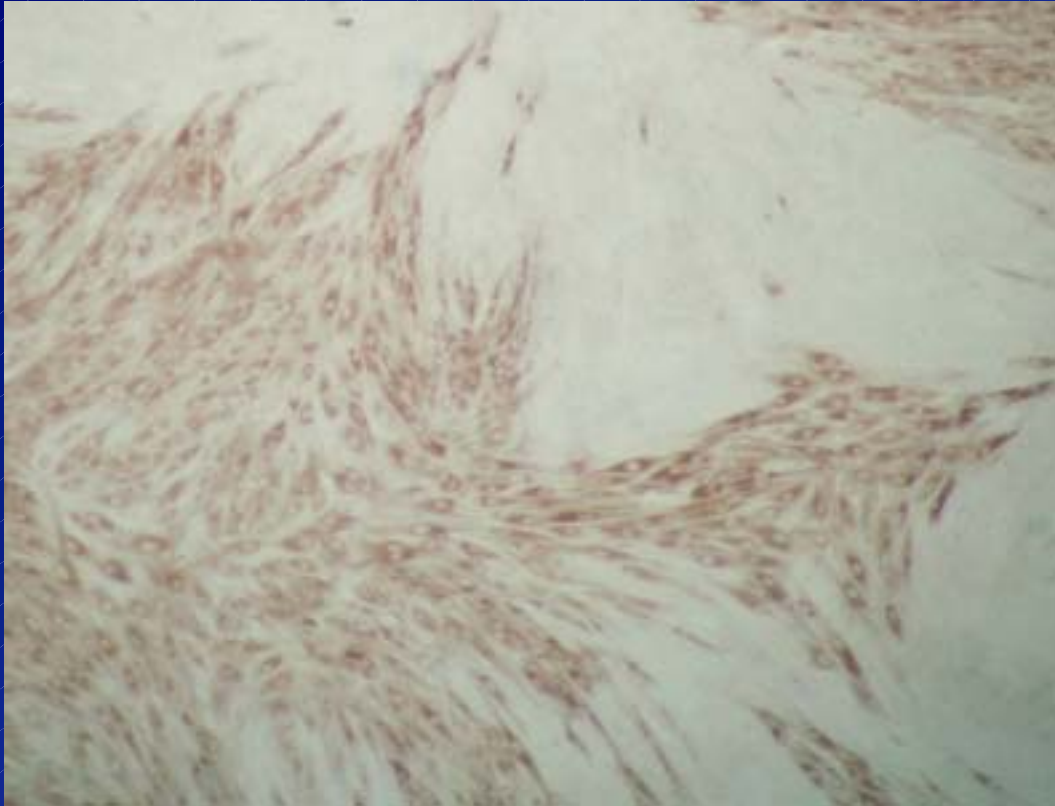


bovine
turbinate cells
-
3 days post
infection with
cp BVDV
-
destroyed
monolayer

IMMUNOPEROXIDASE TEST - IPX

- ◆ *simplified micro-format virus isolation / detection*
 - ◆ single passage culture
 - ◆ fixation of cells
 - ◆ enzyme linked antiserum for visualisation of viral antigens
 - ◆ 4-5 days turnover time
 - ◆ compromise on sensitivity but ideal for detection of PI cattle
- ◆ *may perform well in experienced hands*
- ◆ *used successfully in Scandinavian control programmes*

IMMUNOPEROXIDASE TEST



bovine calf
kidney cells

-
noncytopathogenic
BVDV

-
stained with anti-
NS3 monoclonal
antibodies

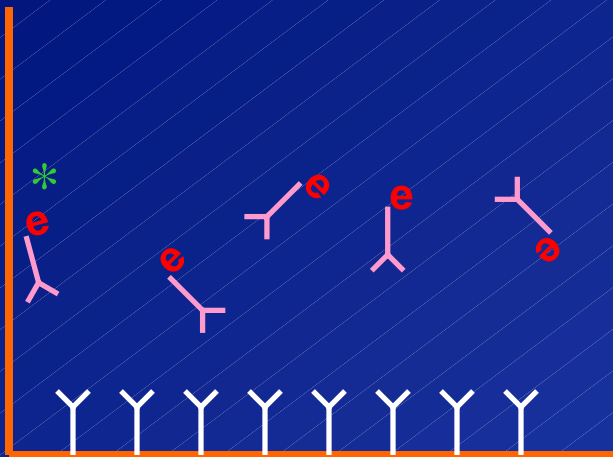
-
viral antigen in
cytoplasm

ANTIGEN ELISAs

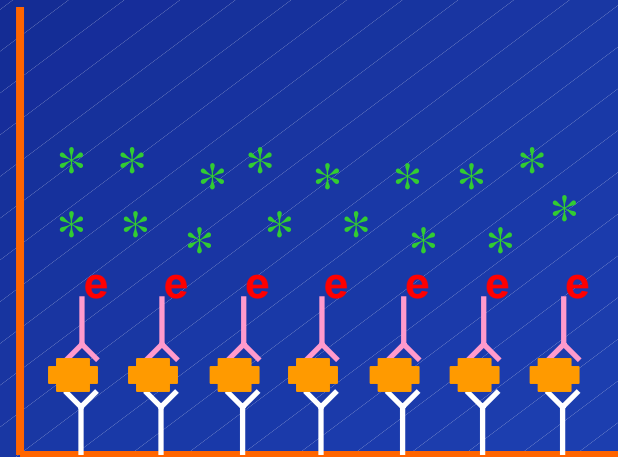
- ◆ same basic principle as for antibody ELISAs
 - ◆ rapid & cell culture independent
- ◆ capture of *preformed* viral antigen
 - ◆ blood or tissue samples
- ◆ usually targets a single viral antigen
 - ◆ NS2-3 or E^{rn}s
- ◆ easier to set up and run than IPX
- ◆ many commercial kits available

ANTIGEN ELISAs - SANDWICH LAYOUT

negative



positive



 detection antibody

 substrate

 capture antibody

 antigen in sample

ANTIGEN ELISAs - OUTPUT

- ◆ OD values - compared to included standard
- ◆ qualitative result
- ◆ inconclusive zone ?
- ◆ interpretation of result
 - ◆ acute or persistent infection ?
 - ◆ *more likely to pick up PI than AI animals*
- ◆ risk of false pos / neg results ?
 - ◆ no morphological recognition as in the IPX

DIFFERENT ANTIGEN ELISAs

- ◆ *NS2-3 detection*

- ◆ high-affinity MABs / -avidity antisera ++
- ◆ genetically conserved - wide detection range ++
- ◆ inhibition by maternal AB in young PI calves -

- ◆ *Erns detection*

- ◆ most conserved immunogenic E-glycoprotein
- ◆ secreted from infected cells - serum/plasma +
- ◆ less influenced by maternal AB inhibition +

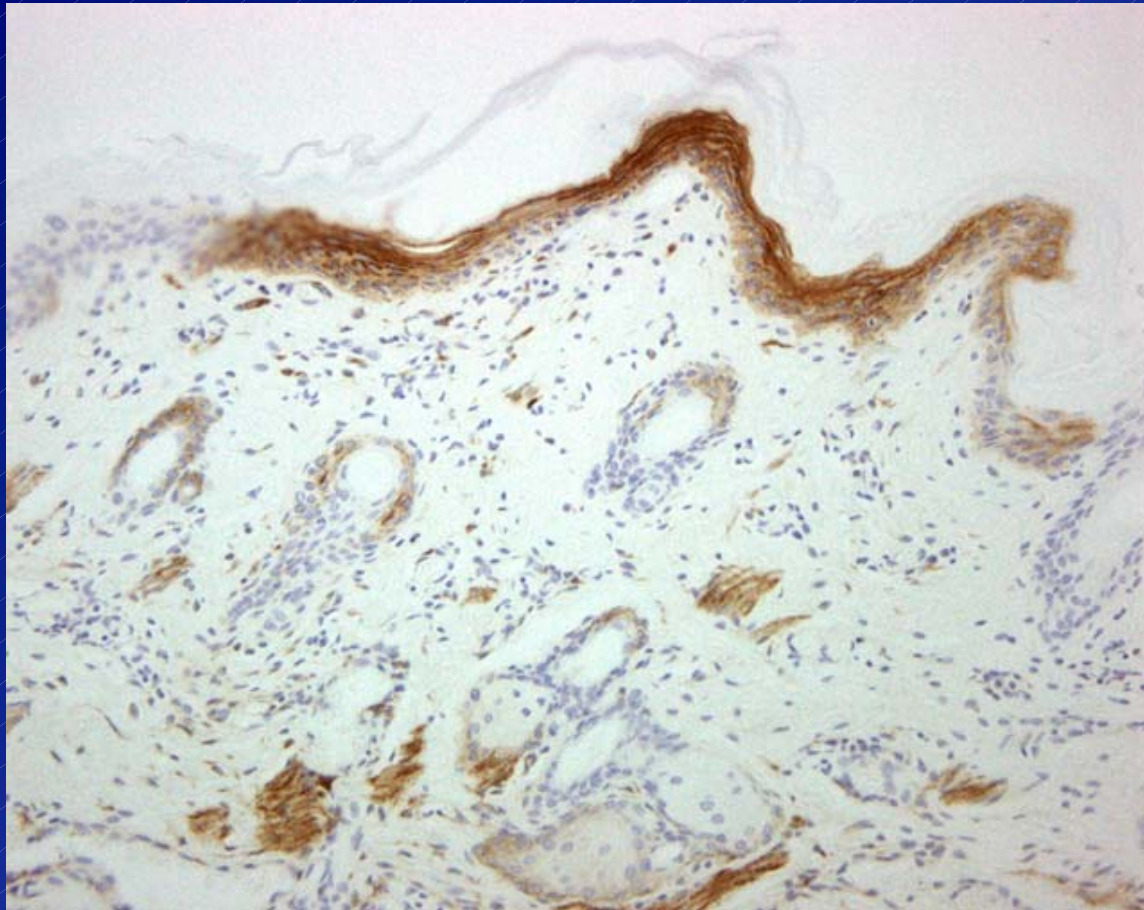
WHICH ANTIGEN ELISA TO CHOOSE ?

- ◆ *in-house or commercial ?*
- ◆ *ask for detailed specifications*
 - ◆ reactivity of MABs /antisera
 - ◆ validation - published data ?
 - ◆ version history ?
 - ◆ solid-phase format ?
 - ◆ convenient in use ?
- ◆ *NS2-3 or Erns ?*
 - ◆ broad reactivity <-> testing of young calves

IMMUNOHISTOCHEMISTRY

- ◆ same as IPX but on preformed viral AG in tissues
- ◆ used for screening of calf skin biopsies
 - ◆ identification of PI animals - young calves
- ◆ requires histopathology-lab (!)
- ◆ immunostaining compatible with tissue fixatives ?
 - ◆ formaldehyde-resistant MAB epitopes
- ◆ convenient approach for vaccinated populations
 - ◆ used successfully in the U.S.A.

IMMUNOHISTOCHEMISTRY



calf skin
section

-

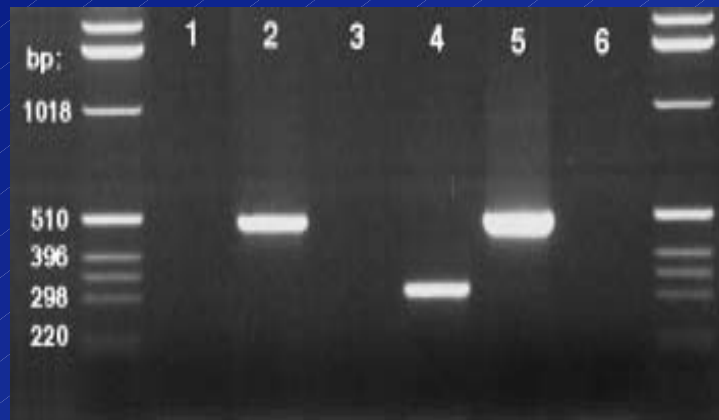
MAB 15c5

-

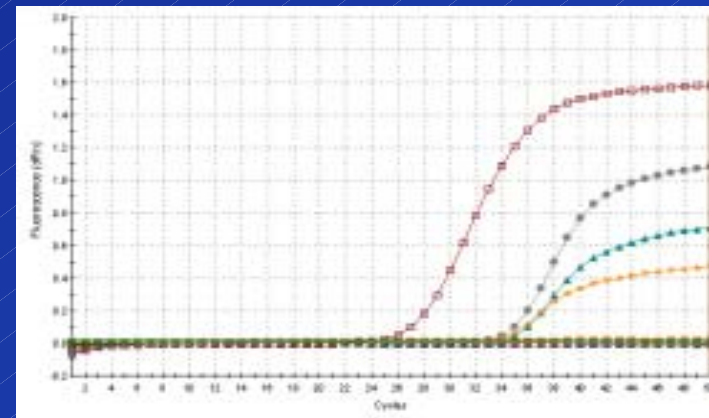
exception to the
rule - skin
section from
acutely infected
calf (10 d p.i.)

RT-PCRs

- ◆ *four steps to detect viral nucleic acid :*
 - ◆ RNA extraction from tissues, blood or milk
 - ◆ reverse transcription to cDNA
 - ◆ primer-directed amplification of specific DNA
 - ◆ visualization of product :



gel electrophoresis



real-time Q-RT-PCR

RT-PCRs

- ◆ *advantages*
 - ◆ very sensitive
 - ◆ rapid . . . + / -
 - ◆ independent of neutralising antibodies in samples
 - ◆ ideal for pooled samples - blood, bulk milk
- ◆ *disadvantages*
 - ◆ expensive equipment
 - ◆ high skill level required
 - ◆ false positive risk - can be too sensitive
 - ◆ primers and probes may be too specific
- ◆ *"in-house" approach*

USE OF RT-PCRS FOR BVDV DIAGNOSIS

- ◆ what is actually detected -
 - ◆ BVDV in PI or also acutely infected animals ?
- ◆ validity of test result
 - ◆ extraction controls
 - ◆ cross-contamination controls
- ◆ is the test result based on representative samples ?
 - ◆ bulk milk - dry PI cows

COMBINATION OF DIAGNOSTIC TESTS

- ◆ *purpose of diagnostic work :*
 - ◆ surveillance
 - ◆ individual animal testing
 - ◆ quality control
- ◆ *choose sets of tests suitable for each level - based on*
 - ◆ BVD epidemiological status
 - ◆ husbandry practices
 - ◆ immunoprophylactic measures
 - ◆ aims of control programmes
 - ◆ resources available

EXAMPLE - BVD SURVEILLANCE

◆ *serology*

- ◆ bulk milk antibody ELISA?
- ◆ spot testing of *cows*
- ◆ spot testing of *young stock*

vaccination compatible?

no

no

yes

◆ *virus detection*

- ◆ bulk milk RT-PCR
- ◆ pooled blood RT-PCR

yes

yes

EXAMPLE - INDIVIDUAL ANIMAL TESTING

a) unvaccinated herds

- ◆ *serology - antibody ELISA*
 - ◆ positive -> no further sampling
 - ◆ negative -> IPX, AgELISA
- ◆ *limitations to virus detection :*
 - ◆ young PI calves with maternal AB
 - ◆ pregnant cows carrying PI foetuses
- ◆ *resolve by:*
 - ◆ test older calves, or use IHC, RT-PCR or E^{rns} AgELISA
 - ◆ semi-quantitative AbELISA, RT-PCR - fetal fluids

EXAMPLE - INDIVIDUAL ANIMAL TESTING

b) vaccinated herds

- ◆ *serology* -
 - ◆ no "DIVA" vaccine / AbELISA designed yet
 - ◆ inactivated vaccine / anti-NS2-3 serology
- ◆ *identifying PI animals* -
 - ◆ IPX *may* give false negative results
 - ◆ AgELISA theoretically better (- inact. vaccines)
 - ◆ RT-PCR unaffected - pooling approach
- ◆ *same precautions for*
 - ◆ young PI calves & PI foetuses

TESTS FOR CONTROL PROGRAMMES

- ◆ *no hard and fast rules !*
 - ◆ know the limits of different test types
 - ◆ obtain sufficient information of commercial kits
 - ◆ plan back-up testing in case of "abnormal" test results
- ◆ IPX ↔ AgELISA → RT-PCR
- ◆ back-up serology to verify all PI animals have been found
- ◆ biosecurity etc. . .

ACKNOWLEDGEMENTS

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