



PIPAViR

HPV E7 oncoproteins as new markers for detection of cervical cancer and precancer

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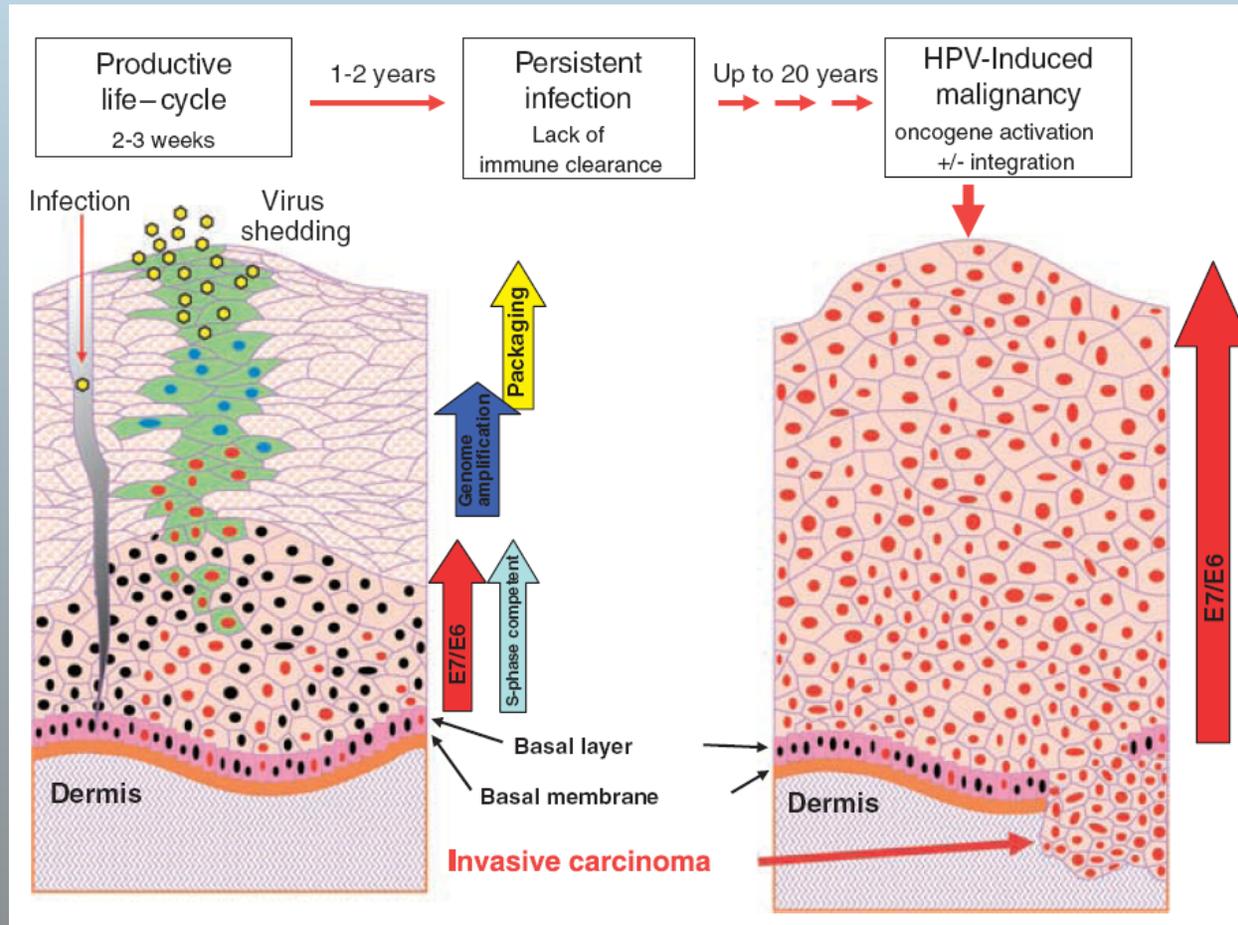
Human Papillomaviruses (HPVs) and cervical lesions

- **HPVs infect basal proliferating epithelial cells of either the skin or mucosa**
- **> 100 different genotypes have been described today**

low-risk types		high-risk types	
HPV 1	skin warts	HPV 5, 8, 9, 12, 14, 15, 17, 19-25	skin cancer
HPV 6, 11	genital warts	HPV 16, 18 , 31, 33, 35, 45 , 58...	cervical cancer

- **cervical cancer is one of the most common cancers in women worldwide**
- **Link between hrHPV infection and cervical cancer firmly established by Harald zur Hausen & colleagues**
- **Nobel Prize in Medicine or Physiology 2008**

Progression from a productive high-risk HPV infection to malignancy



M Thomas et al., Oncogene, 2008

The E6 and E7 oncoproteins are required for tumorigenesis

Current procedures in cervical cancer screening

Detection of abnormal cells

- *Papanicolaou staining* of cells in cervical smears
- **Liquid based cytology**

high rate of false- positive and false- negative results

Detection of viral nucleic acids

- **high- risk HPV DNA**
- **high- risk HPV E6/E7 mRNA**

no discrimination between transient infections and the onset of cervical cancer

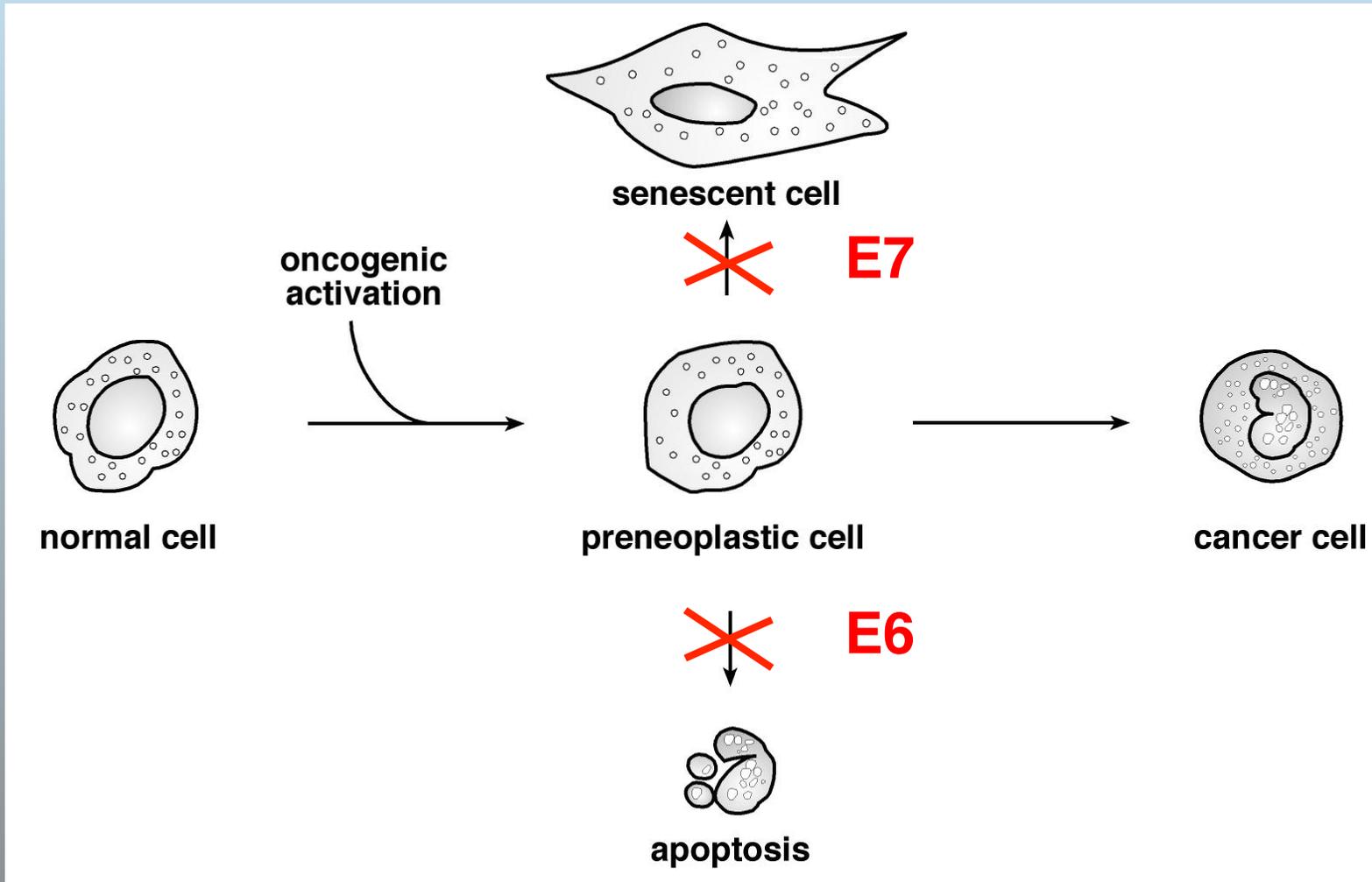
New tools for cervical cancer screening are urgently needed

p16INK4a as surrogate marker for hr HPV infection

- **Cervical swabs of healthy women contain p16INK4a-positive cells**
- **p16/Ki67 double staining ?**

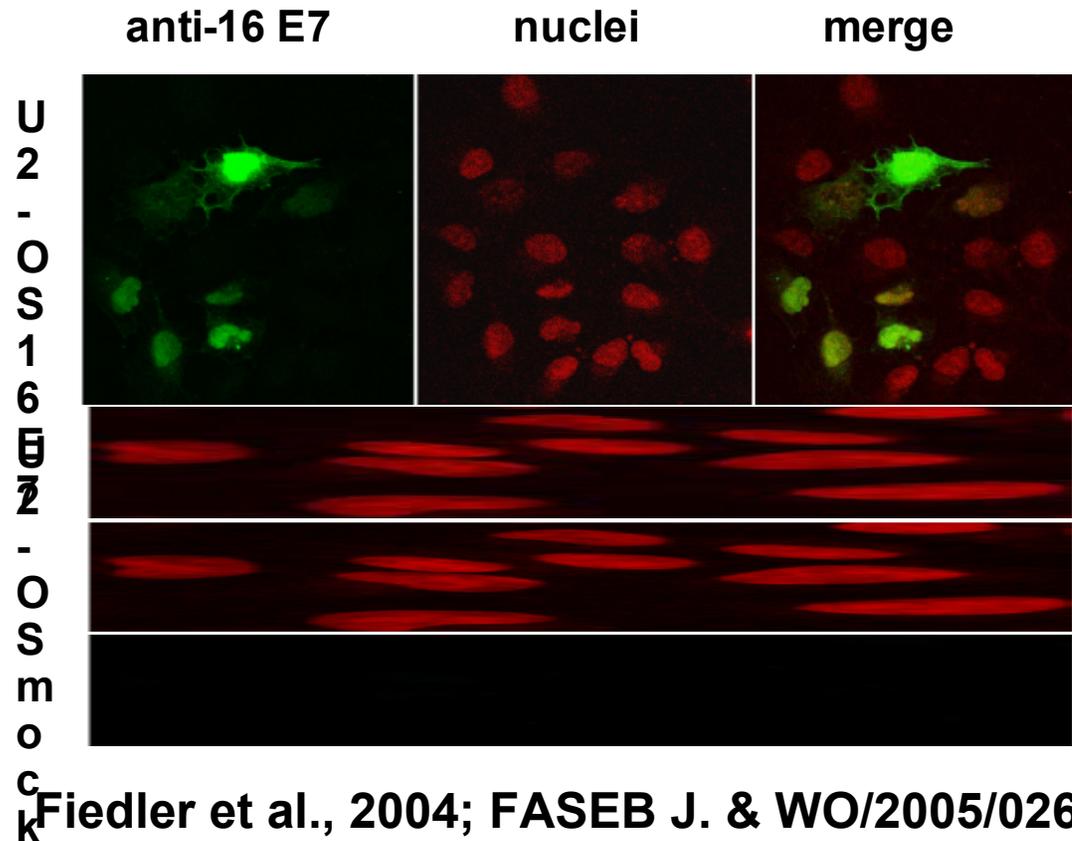
E7 proteins of high-risk HPV types: new markers for cervical cancer?

hrE7 protein: key in cervical carcinogenesis

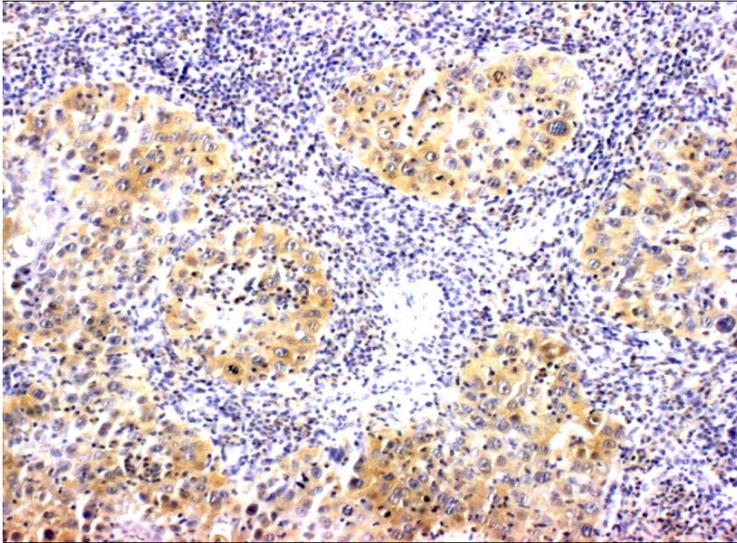


Prediction: E7 protein levels should be high in tumor cells

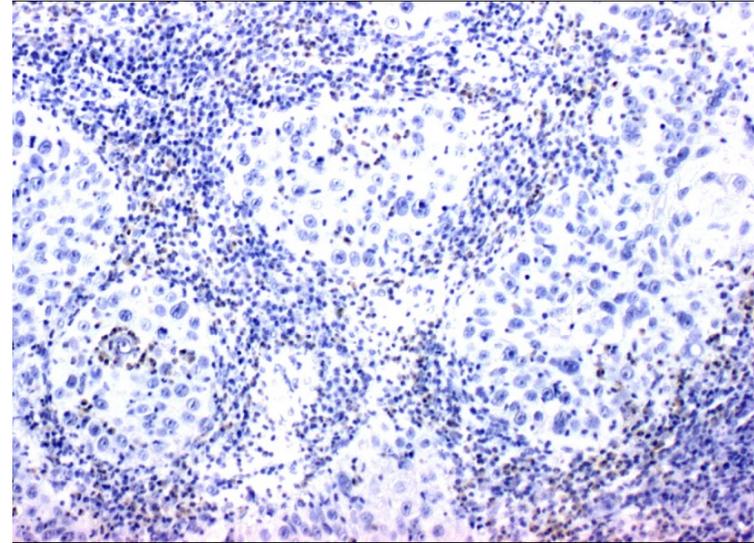
Antibodies to HPV-16 E7 - immunofluorescence



HPV-16 E7 is highly expressed in cervical cancer



**a-HPV-16
E7**



**a-HPV-16
E7/competed**

Fiedler et al., 2004; FASEB J. & WO/2005/026731

Can we use this information for cervical cancer screening?

Viral high-risk E7 proteins: new markers for cervical cancer?

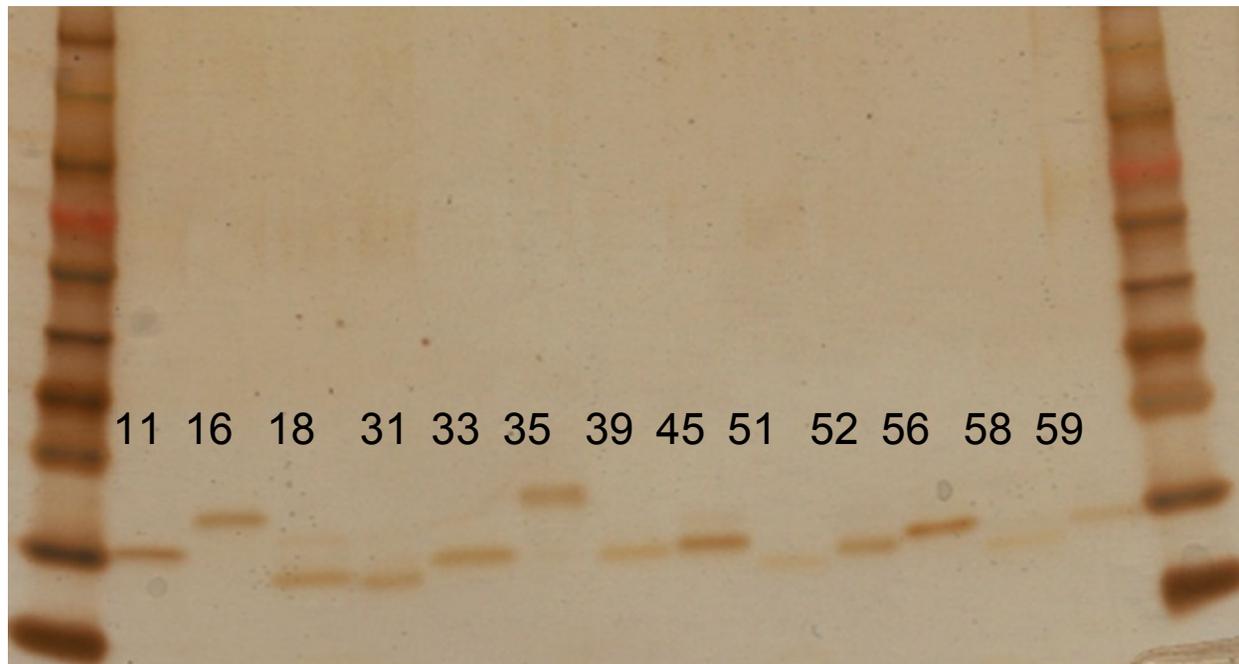
- **E7 protein is necessary to directly inactivate cellular tumor suppressors**
- **HPV-16, HPV-18 and HPV-45 E7 oncoproteins are expressed continuously in CxCa (Ressler et al., 2007; Clin. Cancer Res.) and cervical adenocarcinoma (Dreier et al., 2011; Virology)**

Aim of the study: to detect E7 proteins of hrHPV types in cervical smears

- **Rabbit monoclonal antibodies (RabMabs) against E7 proteins as key diagnostic tools**
- **immunofluorescence detection of E7 proteins in liquid-based cytology (example: RabMab 42-3)**
- **sandwich ELISA to quantitate E7 protein levels in conventional pap smears (example: RabMab 143-7)**

Recombinant E7 oncoproteins

- E7 proteins of the most prevalent hrHPV types were expressed in E.coli and purified
- HPV-11 E7 (low-risk virus) was used as a control



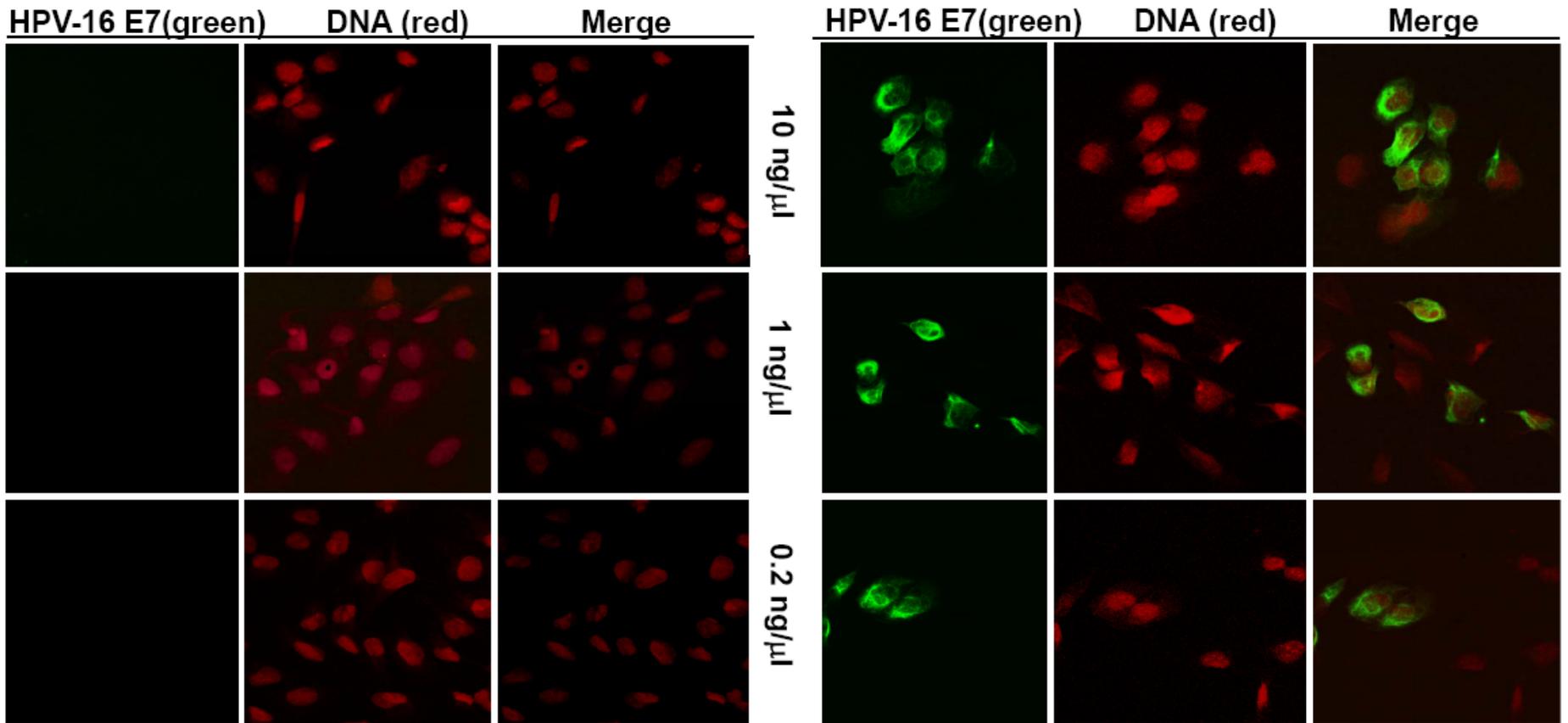
Recombinant E7 proteins (5 mg each) on a silver-stained gel

Rabbit monoclonal antibodies (RabMAbs) for detection of hrHPV E7 proteins

- **E7 proteins of the 12 most prevalent hrHPV types (coverage > 99%) were used to immunize rabbits**
- **Rabbit monoclonal antibodies (RabMabs) produced**
- **> 20 RabMabs were obtained that cover the full spectrum of hrHPV E7 proteins**



Unmatched sensitivity of RabMab 42-3 for IF detection of HPV-16 E7

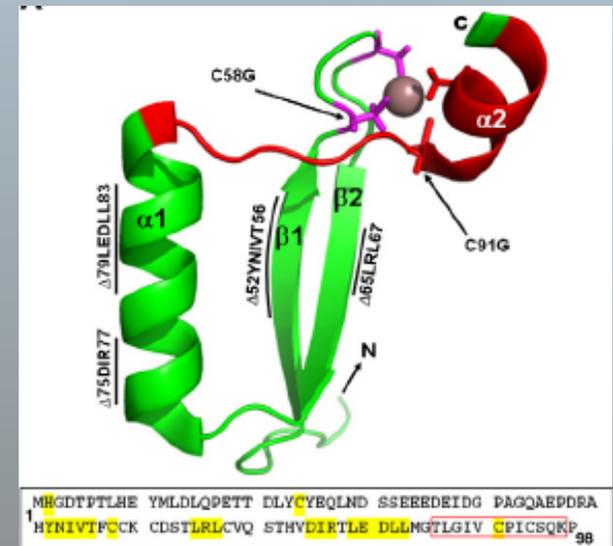
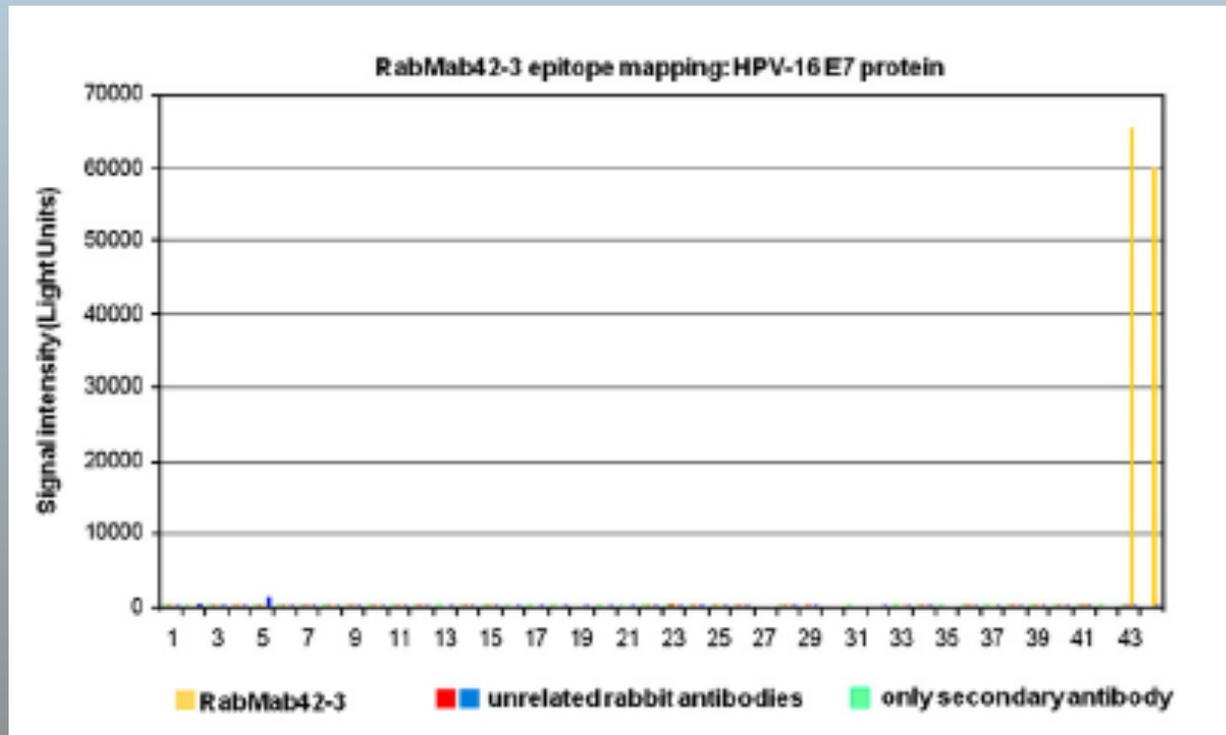


Rabbit polyclonal antibodies
(WO/2005/026731)

RabMab42-3
(WO/2011/101122)

RabMab 42-3 recognizes a conformational epitope in the HPV-16 E7 zinc finger

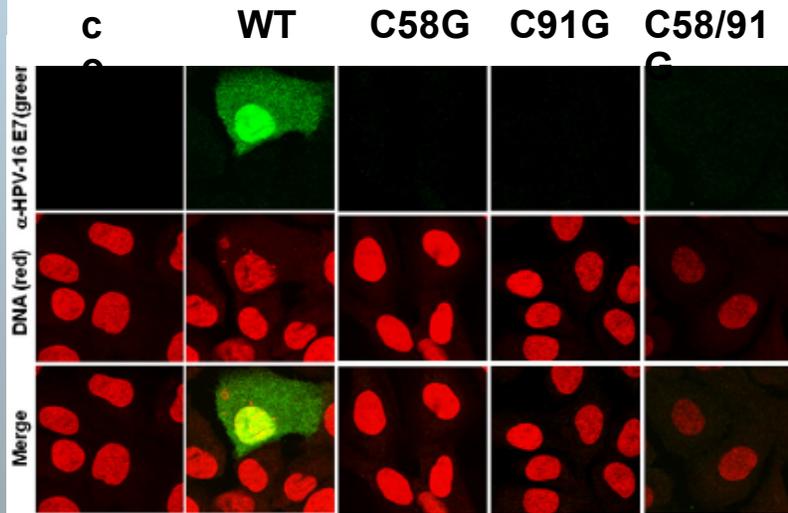
PepScan analysis



Cysteine residues 58/61 and 91/94 stabilize a Zn finger structure in the C-terminal domain of HPV-16 E7

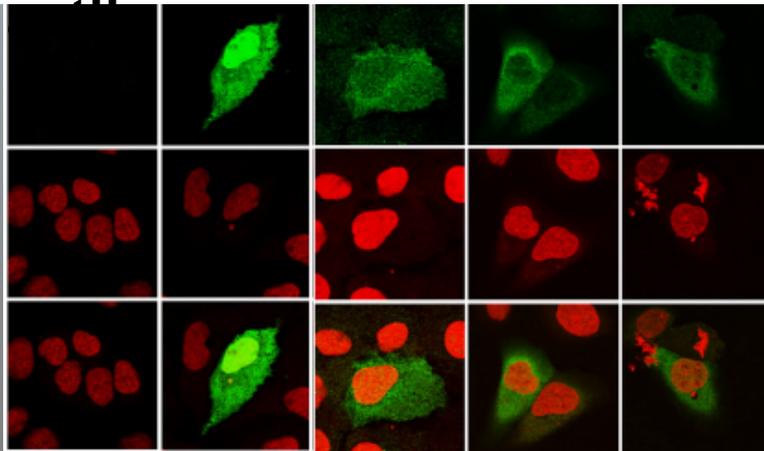
RabMab 42-3 recognizes a conformational epitope in the HPV-16 E7 zinc finger (ctd.)

IF analysis with RabMab 42-

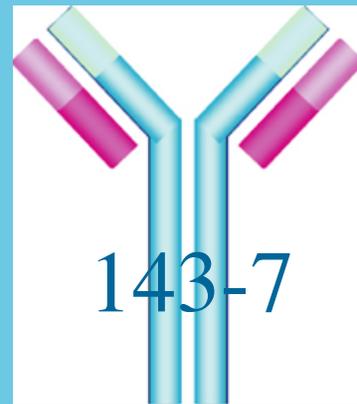


- RabMab 42-3 recognizes an epitope in the HPV-16 E7 C-terminus
- Destabilization of the Zn finger by single Cys mutations abolishes IF signal
- RabMab 42-3 stains endogenous E7 protein in Caski cells (Dreier et al. 2011)
- **LBC analysis underway**

IF with rabbit polyclonal

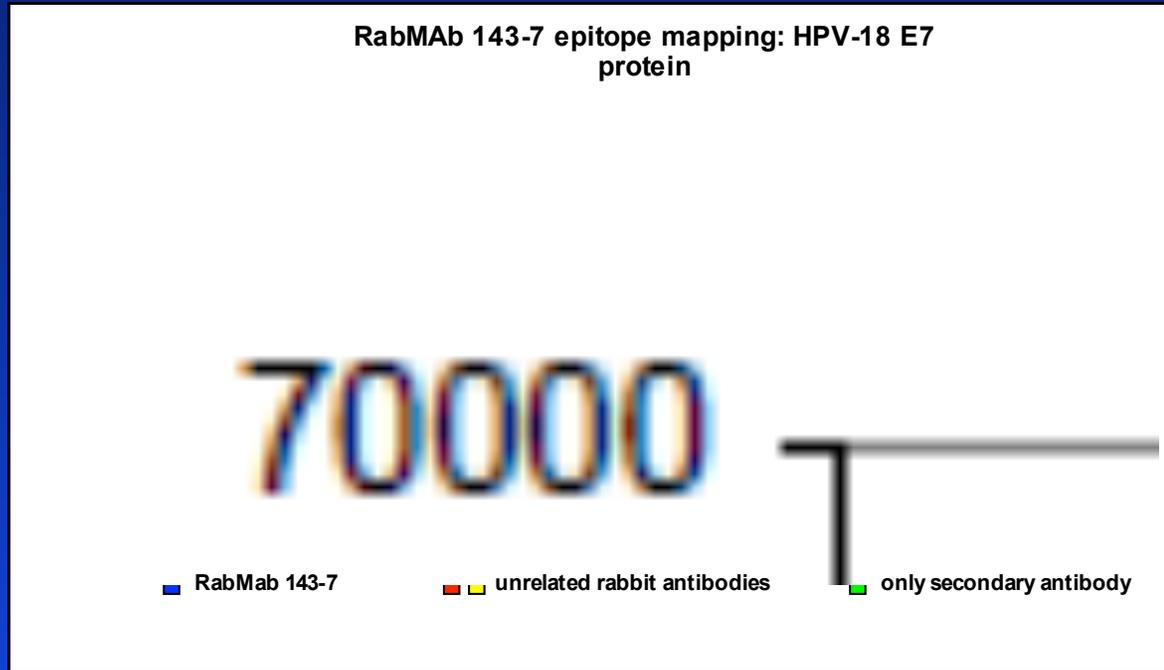


Performance of RabMAb

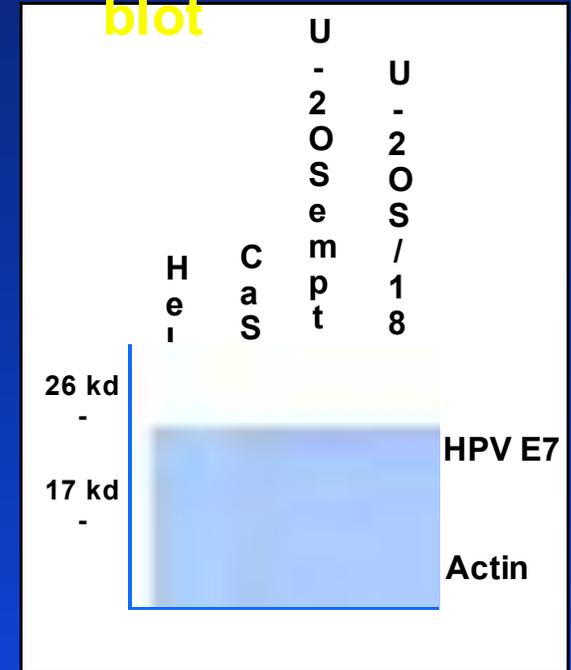


RabMab 143-7 recognizes an epitope in the HPV-18 E7 N-terminus

Pepscan analysis

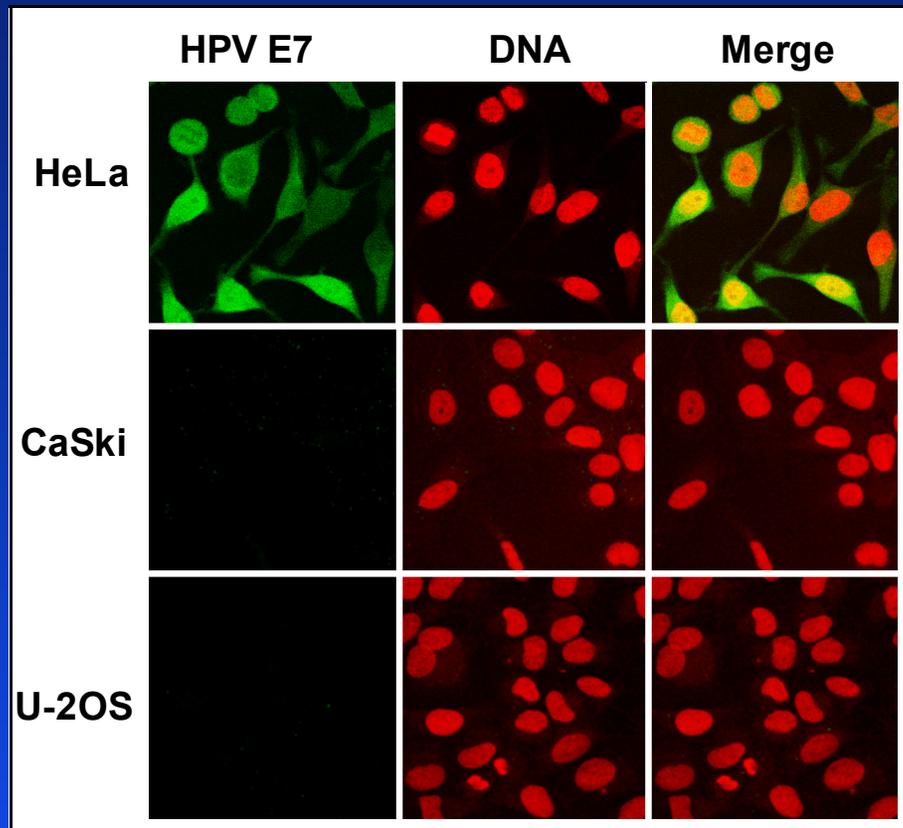


Western blot

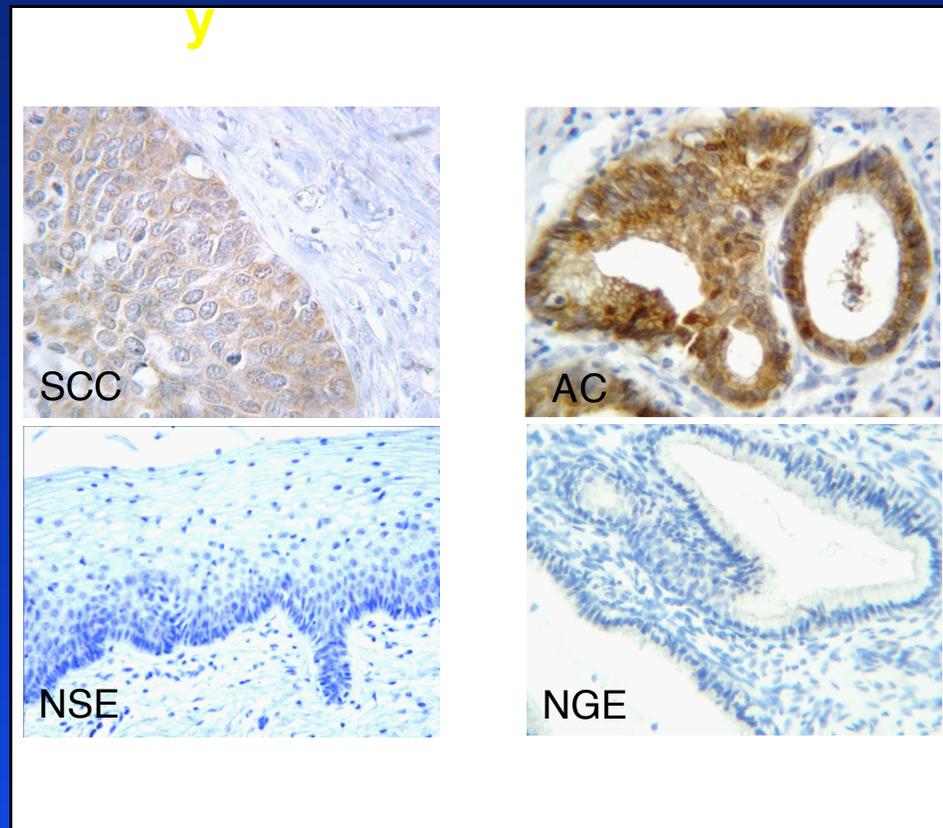


Specific detection of endogenous HPV-18 E7 by immunofluorescence and IHC

Immunofluorescence

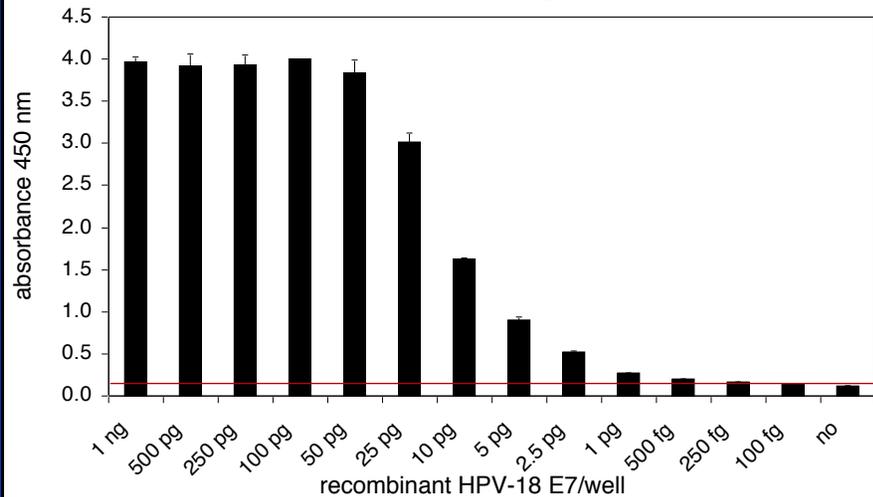


Immunohistochemistry

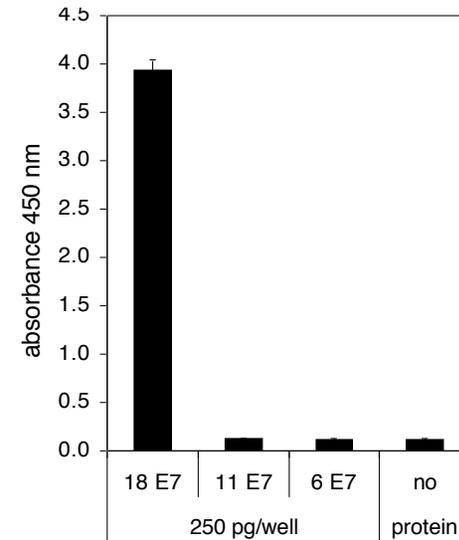


RabMab 143-7 based Sandwich-ELISA

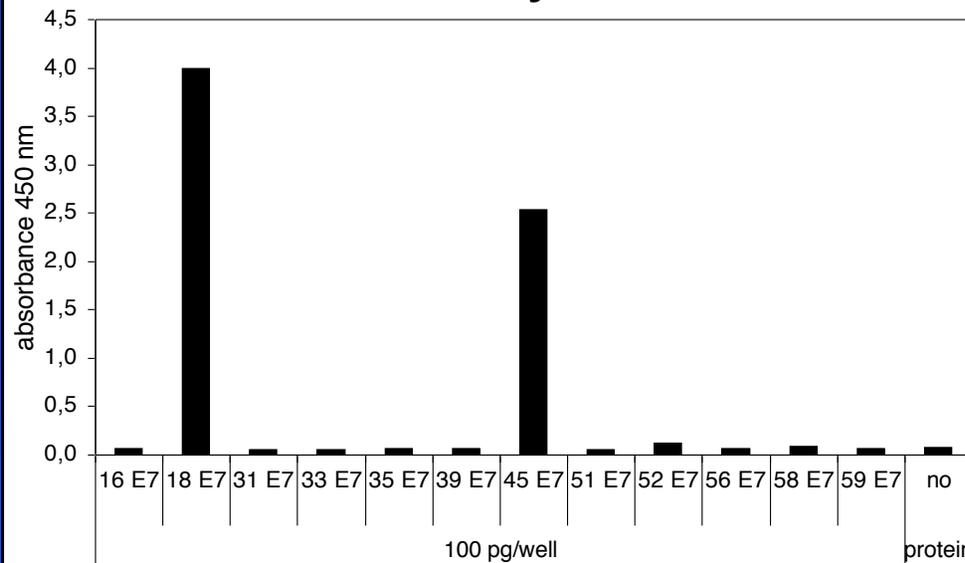
Detection limit < 1 pg 18 E7 protein



low-risk E7 is not detected



Cross-reactivity with HPV-45 E7

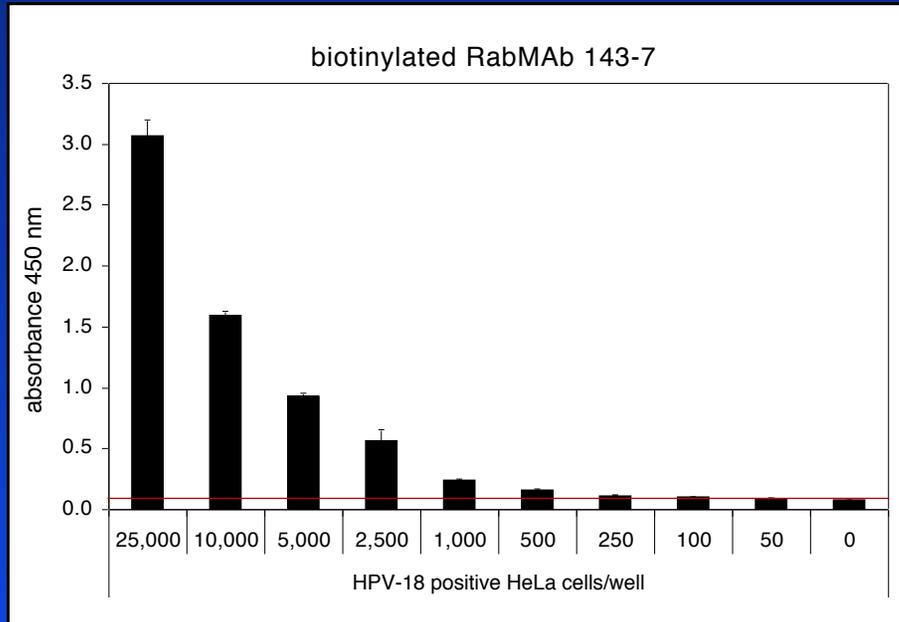


- 143-7-based sandwich ELISA detects recombinant E7 proteins of HPV-18 and -45
- Detection limit < 1 pg

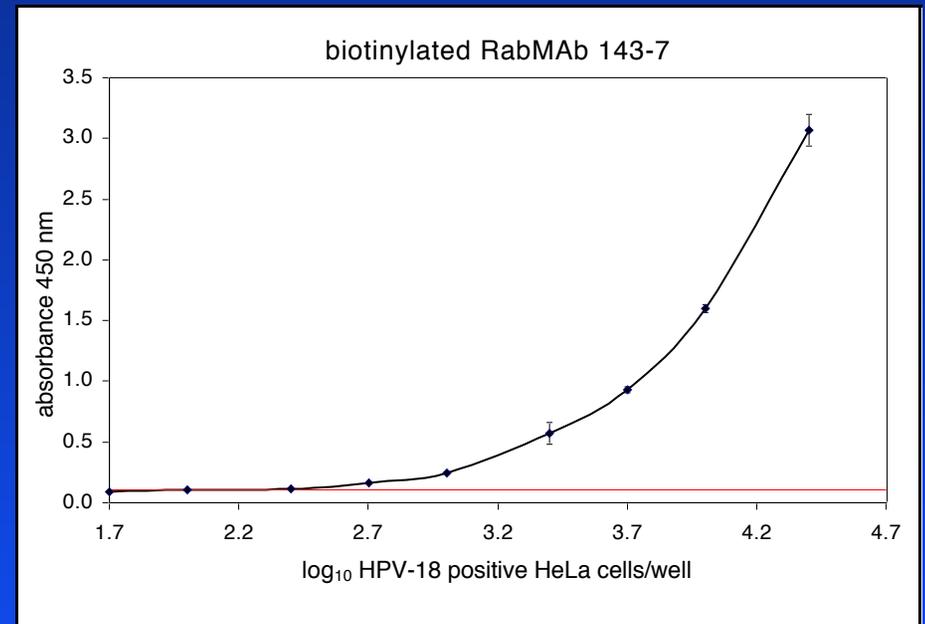
RabMab 143-7 based Sandwich-ELISA (ctd.)

- Decreasing numbers of HeLa cells (HPV-18 positive) added to U2-OS cells (HPV-negative), total cell number 25.000
- Sandwich ELISA with cell lysates to detect HPV-18 E7 protein

HeLa cell titration



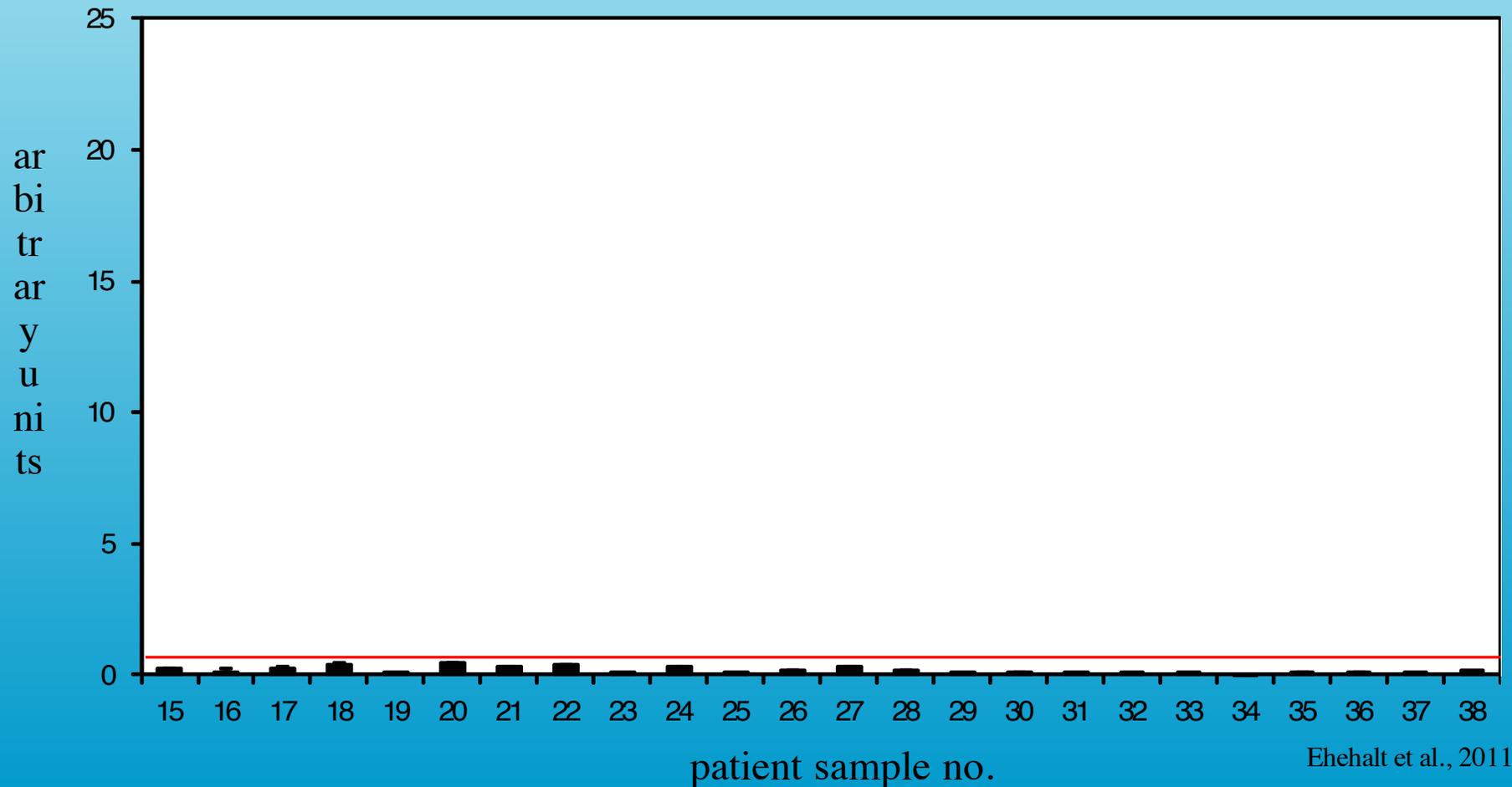
Statistical evaluation (n = 5)



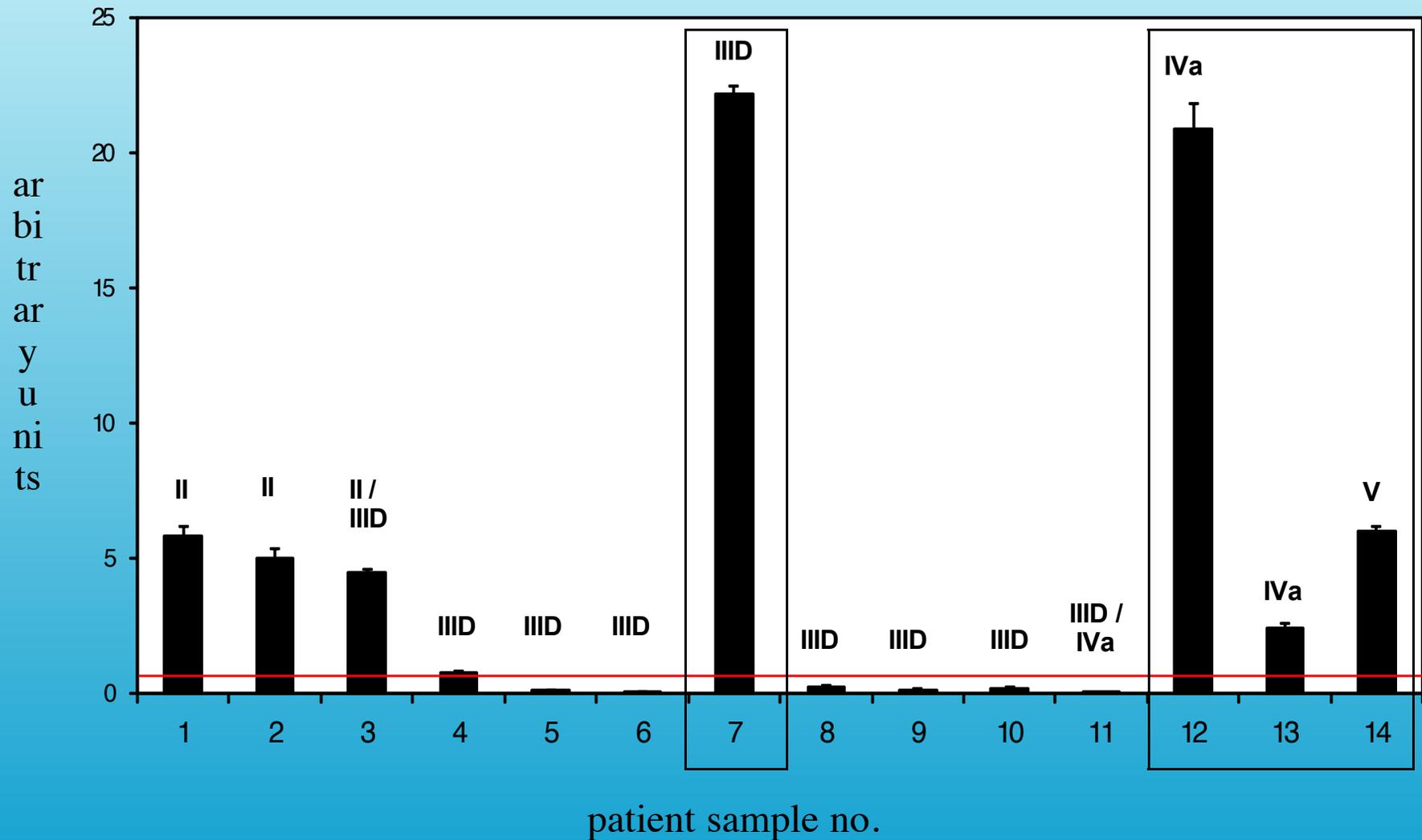
E7 signal from 500 HeLa cells detectable by sandwich ELISA

Pilot clinical study with ELISA based on RabMab 143-7

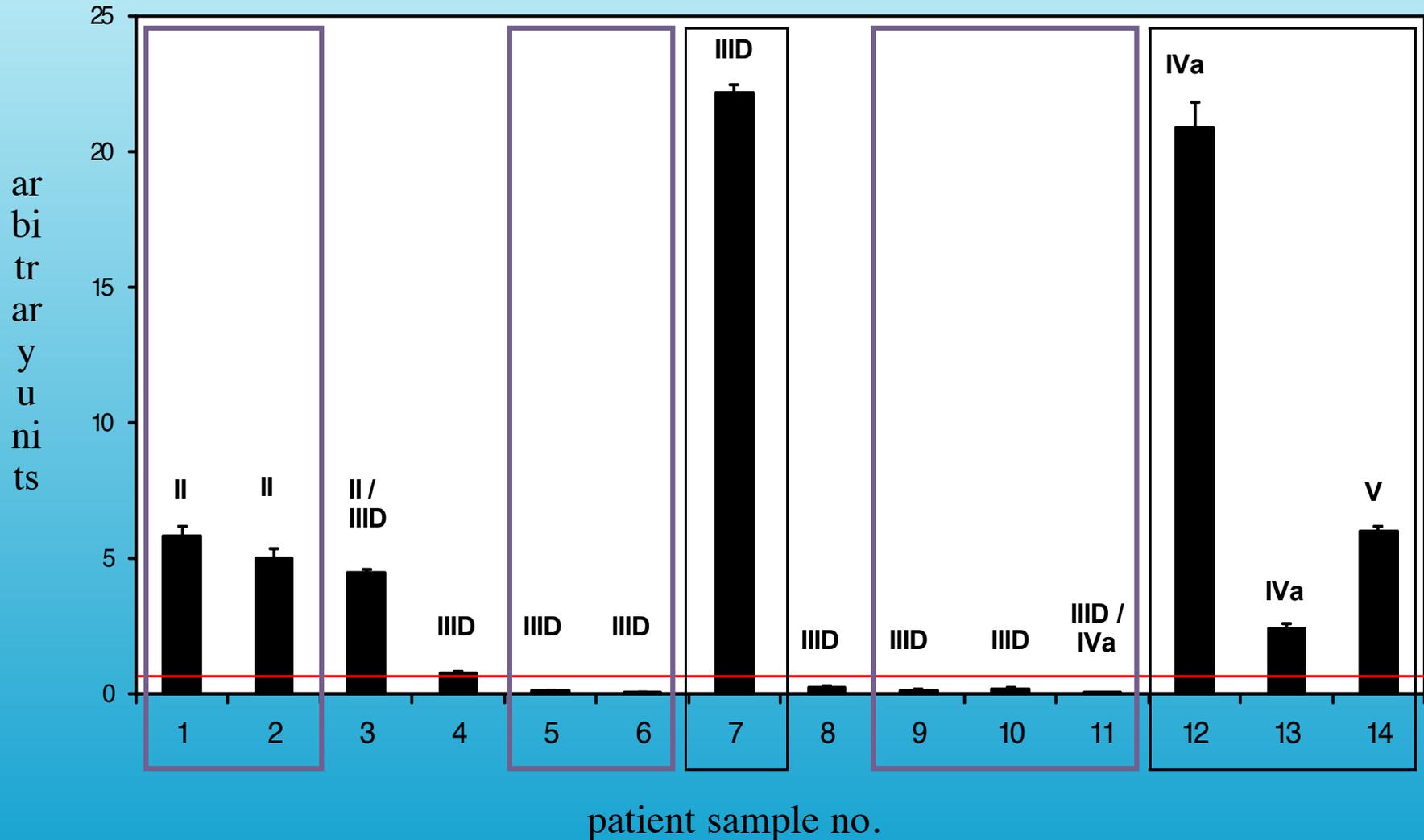
24 HPV- DNA negative +
14 HPV 18- DNA positive cervical
swabs



Pilot clinical study with ELISA based on RabMab 143-7 (ctd.)



Pilot clinical study with ELISA based on RabMab 143-7 (ctd.)



„Problematic“ samples revisited: Cytological diagnosis (PapII) incorrect for patients #1 & #2; multiple hrHPV infections occurred in patients # 5,6,9,10,11

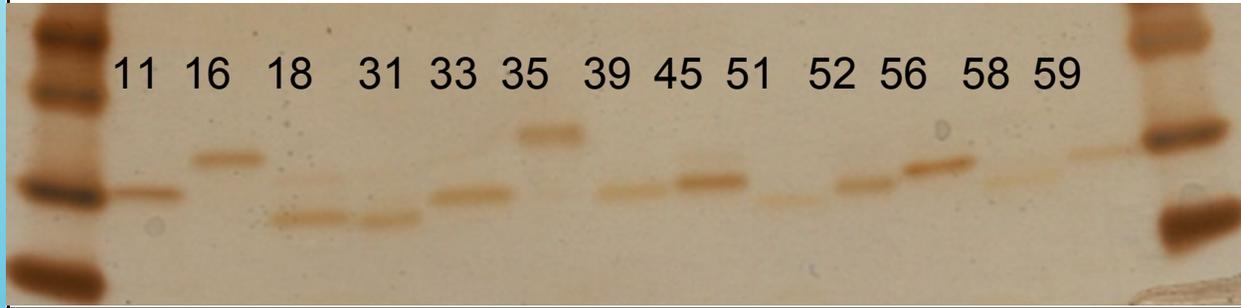


against other hrE7 types

Cross-reactivity of RabMabs towards E7 proteins

Western blot

Purified E7 proteins used in Western blot



RabMab 31-



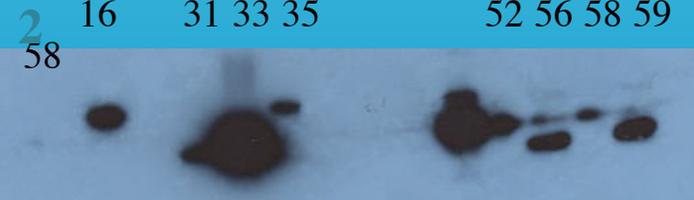
RabMab 38-



RabMab 80-

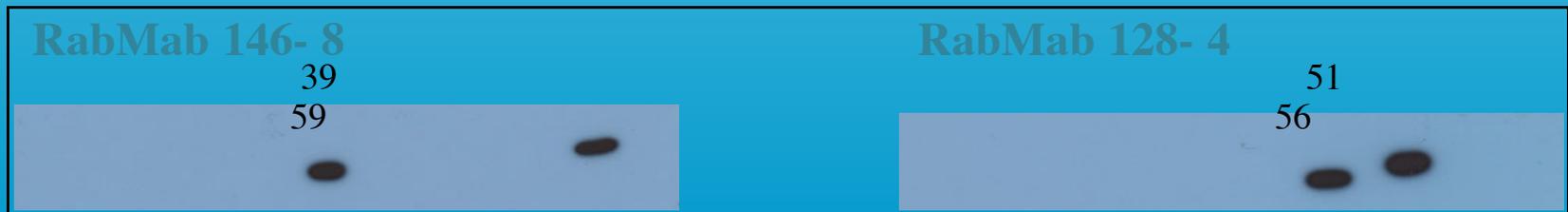
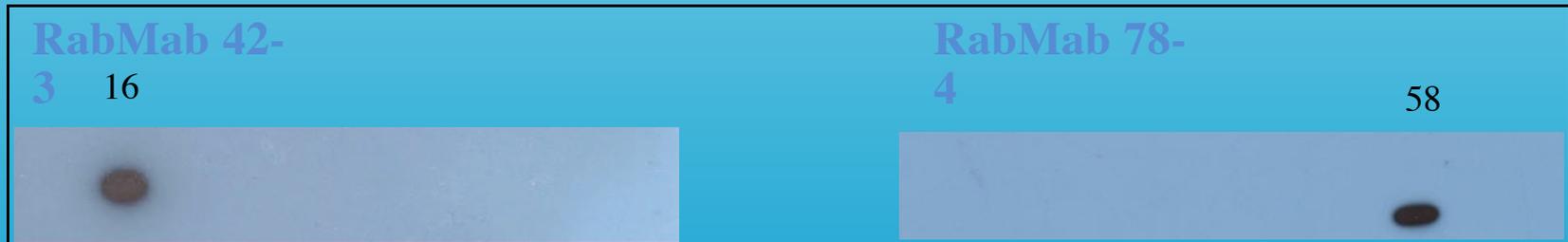
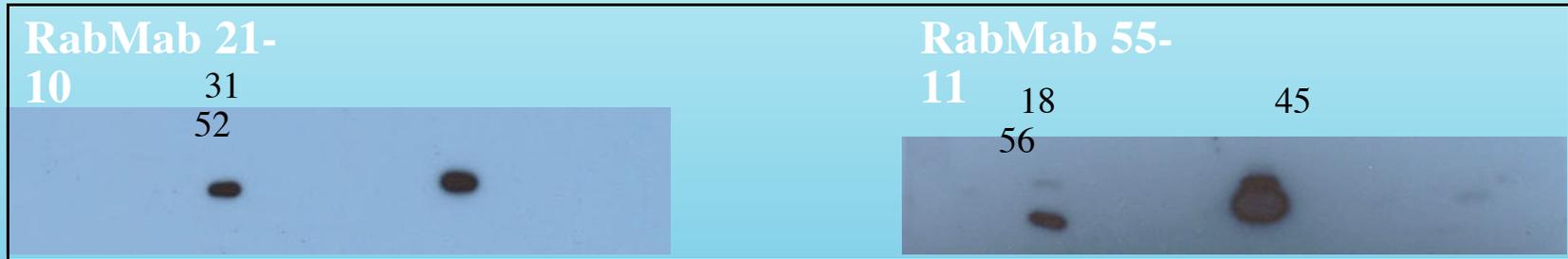


RabMab 84-



Cross-reactivity of RabMabs towards E7 proteins

Western blot (ctd.)

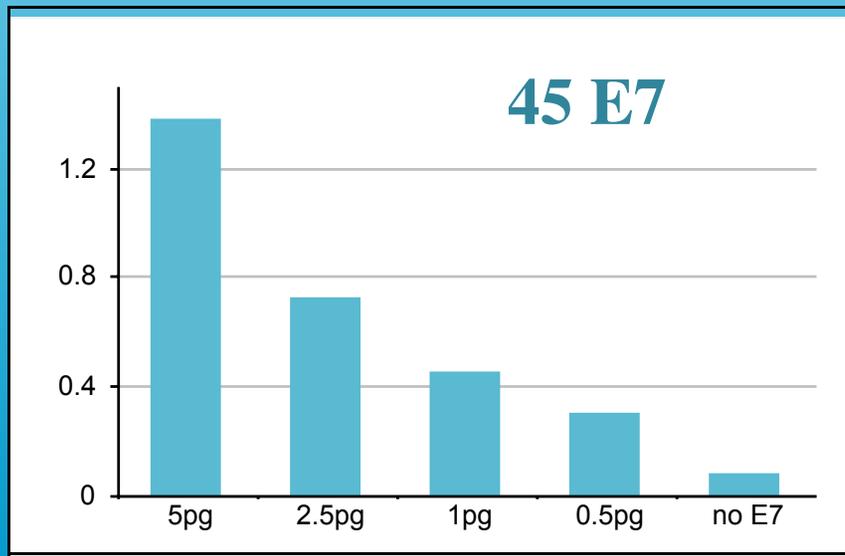
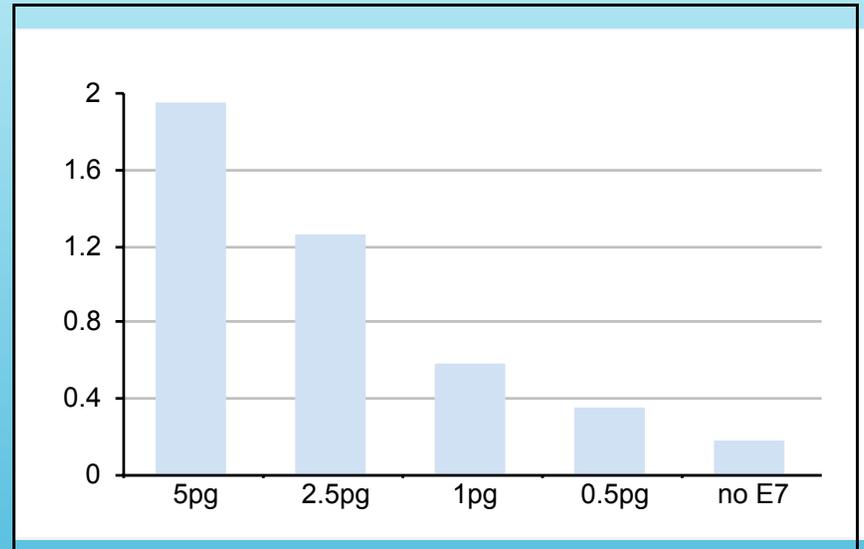
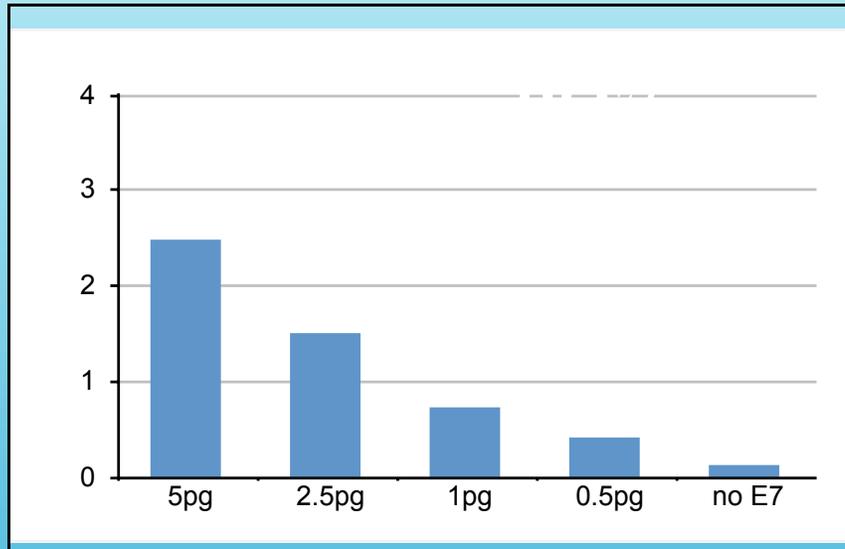


Towards a pan-hrE7 ELISA detection kit

- **RabMabs recognizing other E7 proteins of hrHPV types**
- **Combination of different RabMabs in ELISA plates**
- **Use recombinant E7 proteins to test specificity and sensitivity**

Sandwich ELISA based on RabMab cocktails

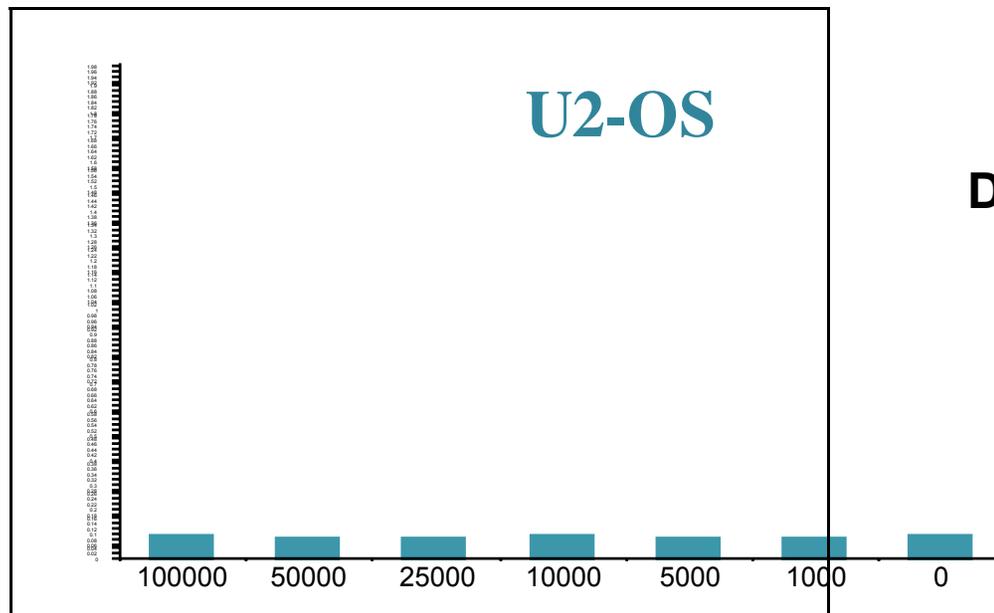
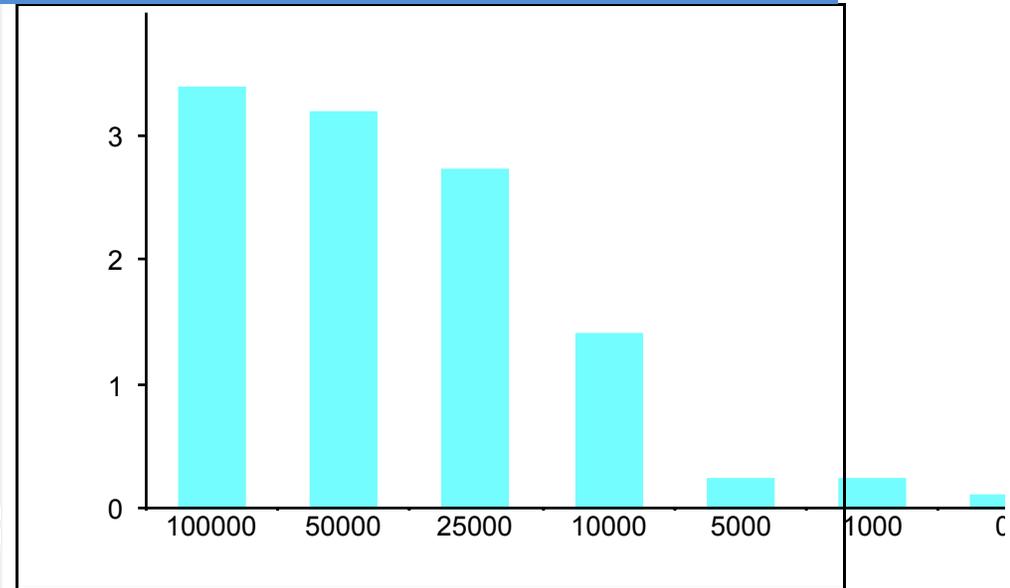
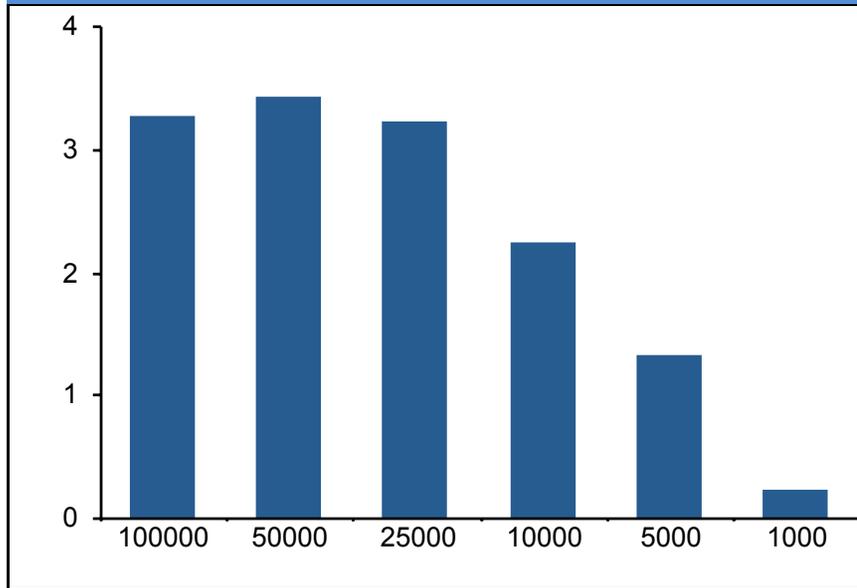
Recombinant E7 proteins



Detection limit: < 500 fg of hrE7 protein

Sandwich ELISA based on RabMab cocktails

Cervical carcinoma cell lines



Detection limit: < 1000 tumor cells

Towards a pan-hrE7 ELISA detection kit

All-in-one E7 detection ELISA

Conclusions

- **E7 proteins of high-risk HPV types as new markers for cervical cancer**
- **Rabbit monoclonal antibodies to hrHPV E7 proteins as key diagnostic tools**
- **sandwich ELISA for E7 detection in cervical smears**
- **Proof of the principle OK, assay optimization underway**
- **Clinical validation underway**

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