HPV Molecular EQAS: Is Quantification Helpful?

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HPV Screening

- Molecular testing for human papillomavirus (HPV) replaced cytology as the screening method for cervical cancer
- HPV testing also recommended in other countries as the preferred screening method

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HPV Screening

- HPV testing helps to identify risk of developing cancer
  - Persistent HPV infection→→Cervical Cancer[1]
- Partial genotyping determines the pathway of treatment for the patient[2]

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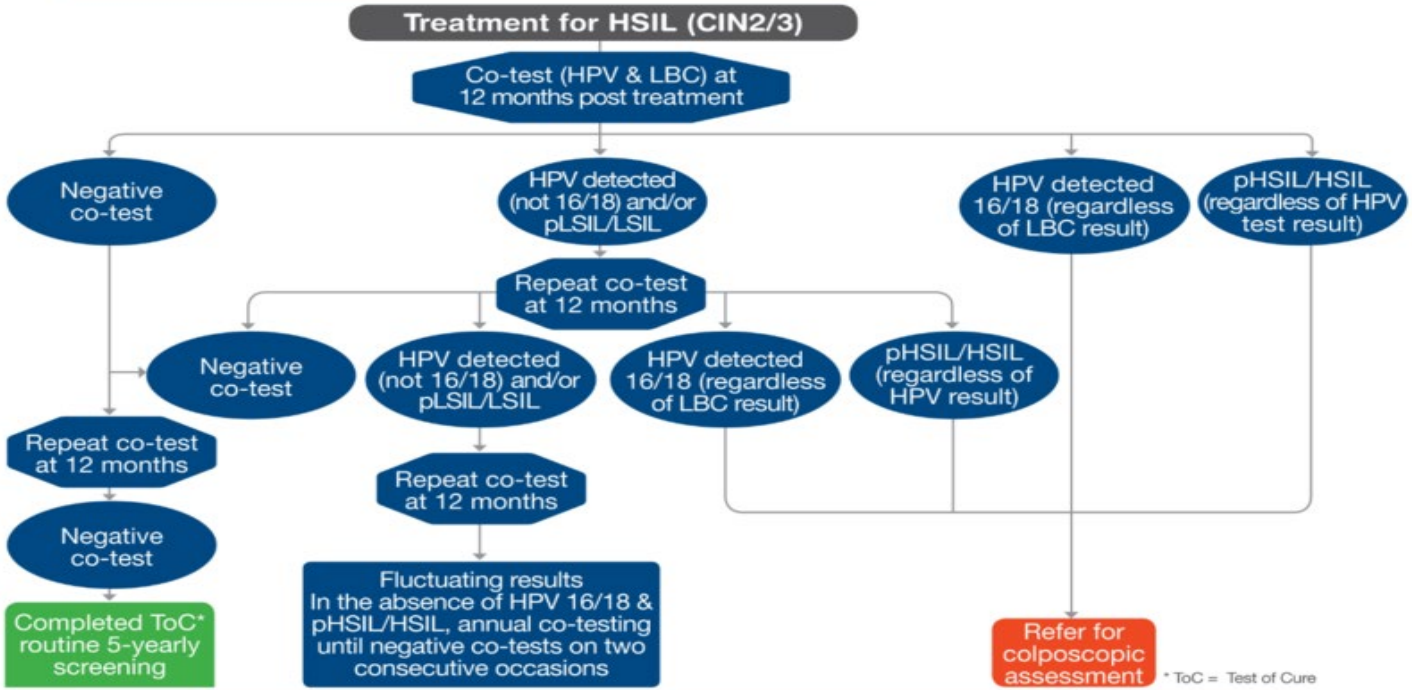
[1]Meijer CJLM et al. *Int. J. Cancer* 2009
[2]National Cervical Screening Program 2017

HPV Screening

TEST OF CURE FOLLOWING TREATMENT FOR HIGH-GRADE SQUAMOUS ABNORMALITIES



Cervical Cancer Screening Guidelines, NCSP, 2017

NRL EQAS for HPV

- NRL released HPVN435 in 2017
- Consists of cultured cells suspended in a Liquid Based Cytology medium
  - HPV positive: types 16 and 18
  - HPV negative: uninfected cells
- Material is quantified using digital droplet PCR (ddPCR)

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Why Quantify?

- Quantified material:
  - provides assay manufacturers and participants “standardised” feedback on overall assay performance
  - allows for critical review of laboratory processes and staff training

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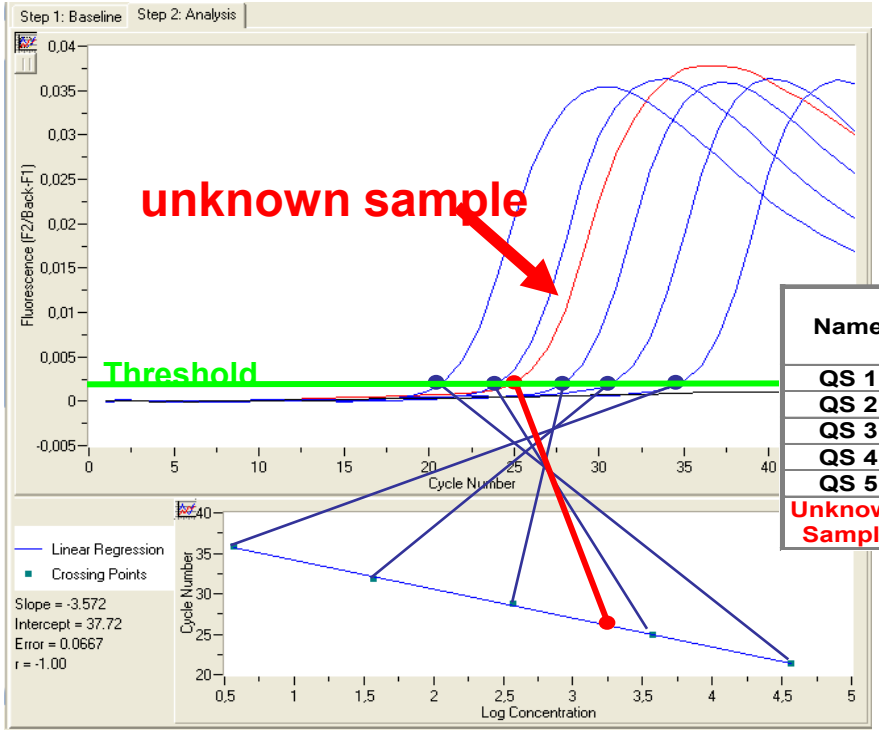
Quantification using qPCR



Name	Crossing point	Standard	Calculated Concentration
QS 1	21.4	$1x10^5$ cop/µl	$1.15x10^5$ cop/µl
QS 2	24.9	$1x10^4$ cop/µl	$1.26x10^4$ cop/µl
QS 3	28.7	$1x10^3$ cop/µl	$0.85x10^3$ cop/µl
QS 4	31.9	$1x10^2$ cop/µl	$1.12x10^2$ cop/µl
QS 5	35.8	$1x10^1$ cop/µl	$0.81x10^1$ cop/µl
Unknown Sample	26.1		$6.21x10^3$ cop/µl

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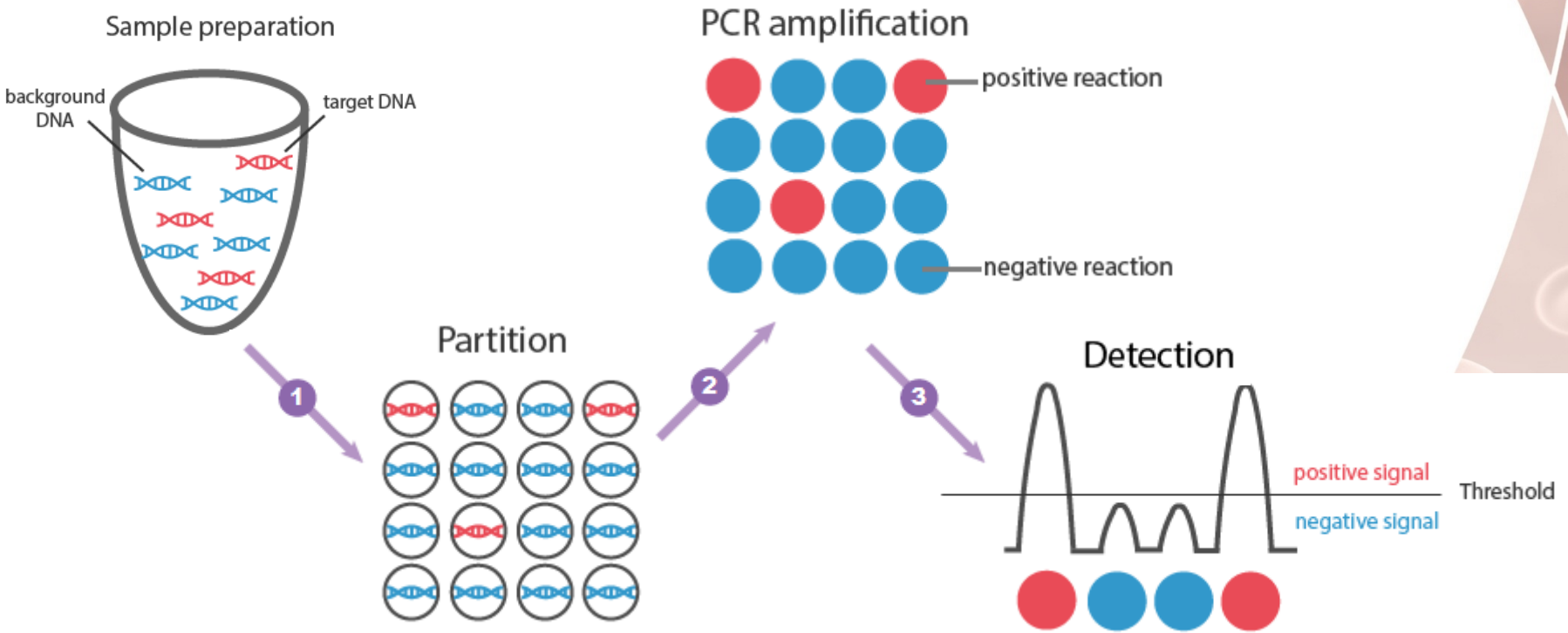
Quantification using ddPCR



https://www.abmgood.com/marketing/knowledge_base/polymerase_chain_variation_system.php

Analysing HPVN435

- Five test events (from two years) were analysed
- Panels distributed to 32 participants from four countries
- Samples were tested in 12 different assays

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Analysing HPVN435

- For submitted results
  - Includes all interpretations
  - Aberrant results are those that are “not concordant” with reference results
- ddPCR allows same concentration samples to be combined in a single analysis

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HPVN435: Panel Configurations

	Year: 2017			Year: 2018	
ID	**TE1**	**TE2**	**TE3**	**TE1**	**TE2**
A	HPV-18 10^4 c/mL	HPV-16 10^4 c/mL	HPV-16 10^5 c/mL	HPV-16 10^4 c/mL	HPV-16 10^4 c/mL
B	HPV-16 10^5 c/mL	HPV-16/18 10^5 c/mL	Uninfected cells 10^4 c/mL	HPV-16 10^4 c/mL	HPV-18 10^4 c/mL
C	Uninfected cells 10^4 c/mL	HPV-18 10^2 c/mL	HPV-16/18 10^5 c/mL	HPV-18 10^4 c/mL	HPV-16 10^2 c/mL
D	HPV-16/18 10^5 c/mL	HPV-16 10^4 c/mL	Uninfected cells 10^4 c/mL	HPV-16/18 10^5 c/mL	Uninfected cells 10^4 c/mL
E	HPV-16 10^4 c/mL	HPV-18 10^4 c/mL	HPV-16 10^4 c/mL	HPV-18 10^3 c/mL	HPV-18 10^4 c/mL

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HPVN435: Panel Configurations

	Year: 2017			Year: 2018	
ID	**TE1**	**TE2**	**TE3**	**TE1**	**TE2**
A	**HPV-18 10^4 c/mL**	**HPV-16 10^4 c/mL**	HPV-16 10^5 c/mL	**HPV-16 10^4 c/mL**	**HPV-16 10^4 c/mL**
B	HPV-16 10^5 c/mL	HPV-16/18 10^5 c/mL	Uninfected cells 10^4 c/mL	**HPV-16 10^4 c/mL**	**HPV-18 10^4 c/mL**
C	Uninfected cells 10^4 c/mL	**HPV-18 10^2 c/mL**	HPV-16/18 10^5 c/mL	**HPV-18 10^4 c/mL**	**HPV-16 10^2 c/mL**
D	HPV-16/18 10^5 c/mL	**HPV-16 10^4 c/mL**	Uninfected cells 10^4 c/mL	HPV-16/18 10^5 c/mL	Uninfected cells 10^4 c/mL
E	**HPV-16 10^4 c/mL**	**HPV-18 10^4 c/mL**	**HPV-16 10^4 c/mL**	HPV-18 10^3 c/mL	**HPV-18 10^4 c/mL**

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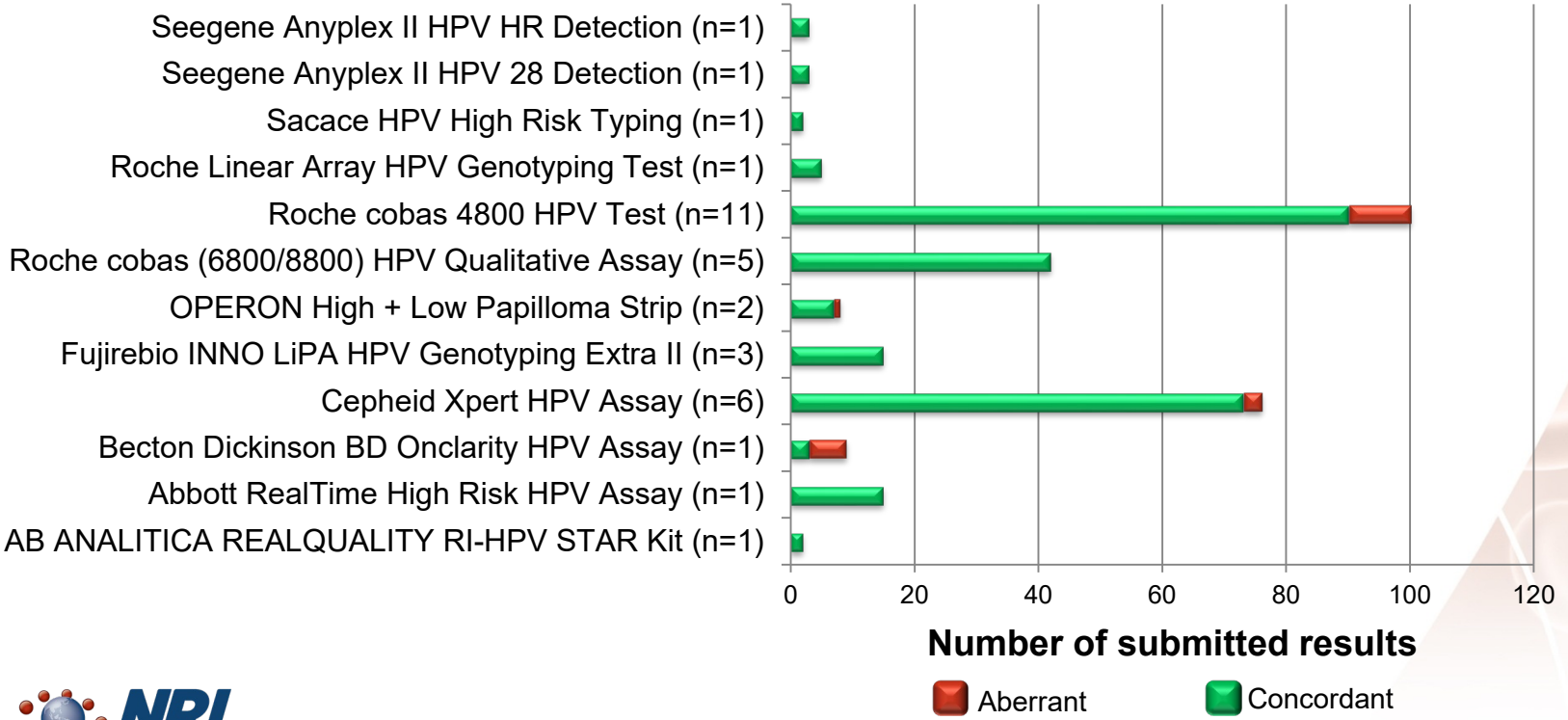
Analysis: HPV-18 at 1x10^4 c/mL



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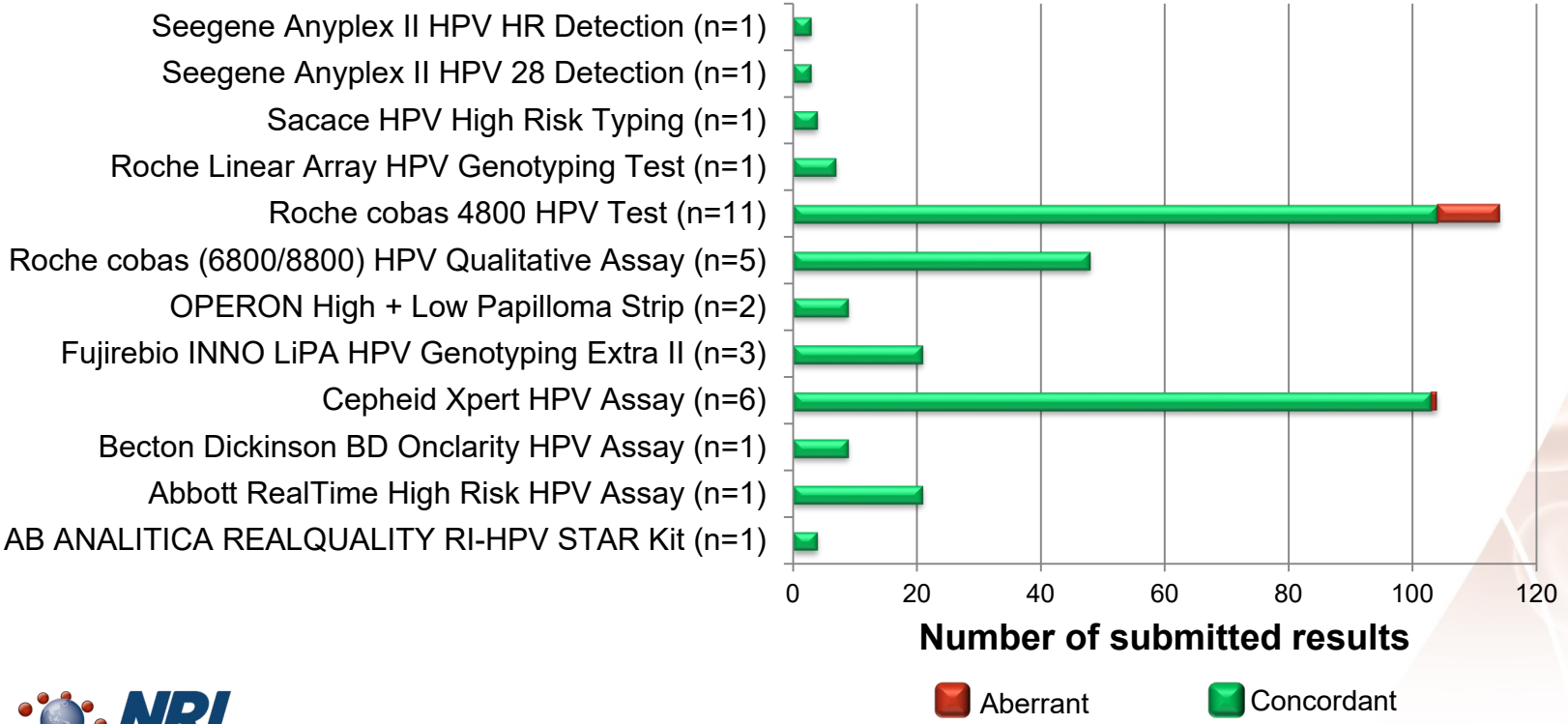
Analysis: HPV-16 at 1x10^4 c/mL



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Detection Rate

Assay Name	HPV-16 1x 10^2 c/mL		HPV-18 1x 10^2 c/mL	
	Aberrant	% Detection	Aberrant	% Detection
AB ANALITICA REALQUALITY RI-HPV STAR Kit (n=1)			0	100
Abbott RealTime High Risk HPV Assay (n=1)	0	100	0	100
Becton Dickinson BD Onclarity HPV Assay (n=1)	3	0		
Cepheid Xpert HPV Assay (n=6)	3	85	0	100
Fujirebio INNO LiPA HPV Genotyping Extra II (n=3)	1	66	0	100
OPERON High + Low Papilloma Strip (n=2)	1	50	1	0
Roche cobas (6800/8800) HPV Qualitative Assay (n=5)	0	100	0	100
Roche cobas 4800 HPV Test (n=11)	0	100	0	100
Roche Linear Array HPV Genotyping Test (n=1)	0	100	0	100
Sacace HPV High Risk Typing (n=1)			0	100
Seegene Anyplex II HPV 28 Detection (n=1)	1	0		
Seegene Anyplex II HPV HR Detection (n=1)	0	100		

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Summary

- Absolute quantification using ddPCR:
  - allowed for combined data analysis
    - benefits smaller peer groups
  - provided useful feedback in assay performance
    - includes assays not available in Australia

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