

UK NEQAS *for Microbiology*

Novos programas de Controlo de Qualidade
Externo: desenvolvimento e perspectivas

15 and 16 October 2008

Biognóstica - Portugal

UK NEQAS

Overview

- Development of new schemes
- Molecular detection of mycobacteria
 - Introduced as a new scheme in 2007
- Molecular detection of HPV
 - Piloted in 2008, for introduction in 2009
- Other schemes in development
 - For introduction in 2009

What are the design criteria for EQA schemes?

- Clinically relevant
- Homogeneous specimens
- No matrix effect
- Stable specimens
- Adequately characterised
- Measurement and assessment of performance is possible

Who makes the decisions?

- Organisers and staff identify possible new schemes after informal discussion with interested parties
- Discussion with potential participants to gain insight into the clinical relevance, the routine approach to testing and possible problems
- Advice from the Steering Committee and Panel on the relevance and approval for pre-pilot studies.

Pre-pilot studies

- Issue a questionnaire to participants to determine common practices and methods and identify those wishing to take part in pilot studies
- Investigate sources of material
- Prepare samples, testing appropriately for homogeneity, stability and performance characteristics in commonly used tests

Pre-pilot studies

- Design request form and instruction sheets
- Design draft scoring schemes
- Draft safety data sheets and risk assessments
- Issue pre-pilot distribution
- Review results, decide on the need for further pre-pilots or progress to the pilot stage

New schemes

Introduced 2007

- CMV DNA quantification
- Antifungal susceptibility
- Molecular detection of mycobacteria

In development

- Measles/Mumps serology
- MRSA screening
- HPV DNA detection.....

Molecular Detection of Mycobacteria Scheme

Summary of distribution results 2007 / 2008

Specimen	Distribution	Result	Correct Direct	% Correct Direct	Correct Post Culture	% Correct Post Culture	Number Testing Rifampicin
8399	2181	MTBC (<i>M. tuberculosis</i>)	53/53	100	43/43	100	18
8400	2181	Negative	50/52	96.2	12/14	85.7	N/A
8527	2218	MTBC (<i>M. tuberculosis</i>)	58/58	100	48/49	98	18
8528	2218	MOTT (<i>M. kansasii</i>)	43/52	82.7*	38/41	92.7*	N/A
8655	2255	MTBC (<i>M. tuberculosis</i>)	63/63	100	47/48	98	32
8656	2255	MOTT (<i>M. avium intracellulare</i>)	52/58	90†	45/47	96†	N/A

MTBC Mycobacterium tuberculosis complex

MOTT Mycobacteria other than tuberculosis

* 'Correct' includes reports of *M. kansasii*, MOTT, and MTBC not detected

† 'Correct' includes reports of *M. avium intracellulare*, MOTT, and MTBC not detected

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Distribution 2181 typing results: Specimen 8399, *M. tuberculosis*

- **MIRU**
 - 8 participants reported results
 - 7 correlated
 - 1 discordant at loci 4, 10

MIRU Type	2	4	10	16	20	23	24	26	27	31	39	40	N° participants
	2	2	8	2	2	5	1	1	3	2	2	2	4
	2	2	8	2	2	5		1	3	2	2	2	1
	2	3	7	2	2	5	1	1	3	2	2	2	1
		2	8	2				1		2		2	1*
		2						1				2	1†

*Participant uses the 15 MIRU-VNTR loci described by Supply *et al.* 2006. JCM. **44** (12):4498-4510.

† Participant in process of setting up MIRU technique

Distribution **2181** typing results: Specimen **8399**, *M. tuberculosis*

- **ETR**

- 6 participants reported results
- 5 correlated
- 1 discordant at locus D

ETR Type	A	B	C	D	E	N° participants
	4	2	4	3	2	4
	4	2	4	2	2	1
	4		4		2	1*

*Participant uses the 15 MIRU-VNTR loci described by Supply *et al.* 2006. JCM. **44** (12):4498-4510.

- **Spoligotyping**

- 5 participants reported results
- 4 gave identical octal designation 775737777420771
- 1 differed at three locations 7757**2733**7420771

- **RFLP**

- 3 participants performed RFLP and 2 submitted images

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Summary

- Performance for direct detection of MTBC was excellent: 100%
- Performance for post-culture detection of MTBC was excellent: 98-100%
- Performance for direct detection of MOTT was good: 82.7-90%
- Performance for post-culture detection of MOTT was good: 92.7-96%
- MIRU and ETR discordant results appear to be due to how participants detect and report partial repeats.
- Maximum of nine participants report MIRU results
- Seven participants report ETR results
- Four participants carry out spoligotyping
- Only two participants carry out and submit RFLP images

The scheme provides specimens characterised such that laboratories performing 'reference' tests are able to compare their performance with other 'reference' laboratories.

Developing the HPV scheme



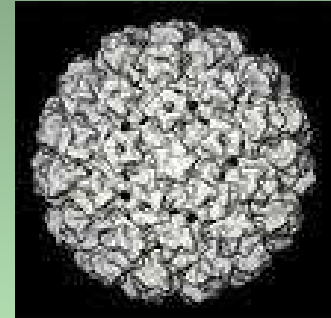
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Background

- HPV belongs to the family Papillomaviridae
- Small, non-enveloped icosahedral viruses
 - 55nm diameter
- Genome: circular, dsDNA, ~8kb
 - Encodes 7 early proteins (E1-7)
 - E1-5 involved in replication & transcription of genome
 - E6-7 oncogenes
 - 2 late proteins (L1-2) - structural capsid proteins



Background (2)

- >100 genotypes of HPV
- >25 cause genital warts
- >15 cause skin warts
- HPVs can also cause warts in the mouth, respiratory tract
- HPVs are specific for humans & the tissues infected, causing either cutaneous or mucosal lesions.



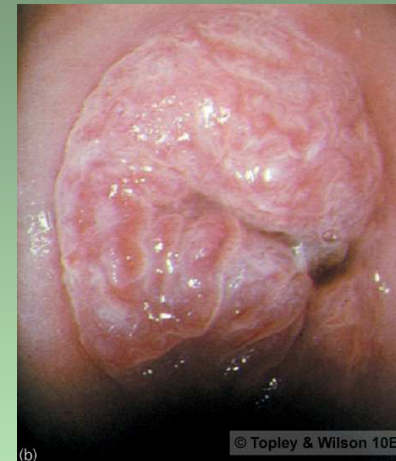
Common warts HPV 2, 27
(<http://vasculitis.med.jhu.edu/treatments/images/warts.jpg>)



Plantar warts HPV 1:
(<http://plantar.org/foot-plantar-wart.jpg>)

Genital HPV Infection

- Divided into 2 categories
 - high-risk (HR): oncogenic, cancer-associated
 - low-risk (LR): non-oncogenic
- HPV 16 & 18 - most common HR types found in cervical cancer
 - Responsible for 70% of cervical cancers



High-grade intraepithelial neoplasia and invasive carcinoma of the cervix
(Topley & Wilson 10th ed. Microbiology & Microbial Infections. Virology:1. p.466. Hodder Arnold: London.)

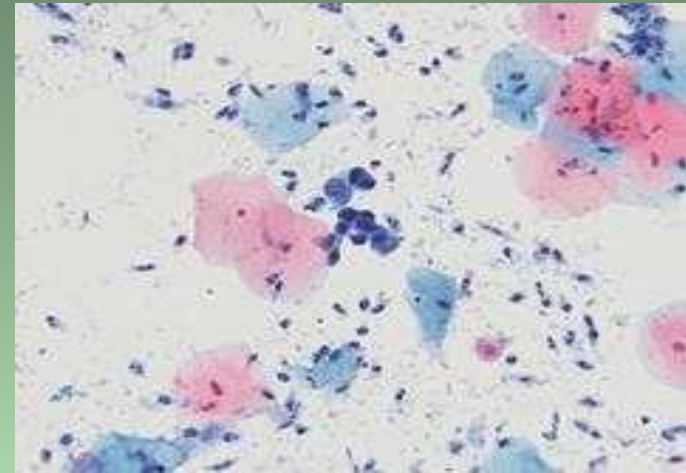
Screening

- 1988: NHS Cervical Cancer Screening Programme
- Method: Cytology
- HPV cervical screening pilot study - looking to add HPV testing to improve effectiveness of screening for early id of cervical cancer.
- Treatment: no cures for HPV, up to the immune system to fight it off
- Prevention: 2 prophylactic vaccines available
 - Gardasil (Merck): HPV 16, 18, 6, 11
 - Cervarix (GSK): HPV 16, 18

Molecular HPV EQA Scheme

Pilot distribution results

- Four cervical specimens in PreservCyt were dispatched with a request for the molecular detection of human papilloma virus (HPV).
- Fifty-nine sets of specimens were sent out and 50 reply forms were received.
- Forty-six participants returned results.
- One lab responded to say they had stopped testing for HPV, another informed us that they had not yet validated their assay and two labs replied that they did not test PreservCyt samples.



Participant responses

Specimen 8772

- Pooled HPV high risk positive sample (genotypes 31,53)
- 93% of participants correctly detected HPV high risk genotypes
- Three participants did not detect high risk genotypes and one reported an indeterminant result.
- All four of these participants used different methods.

Specimen 8773

- Pooled HPV high risk negative sample
- 97.7% of participants did not detect HPV genotypes in this sample.
- One participant reported detecting genotype 66 using the Innogenetics InnoLipA assay.

Participant responses

Specimen 8774

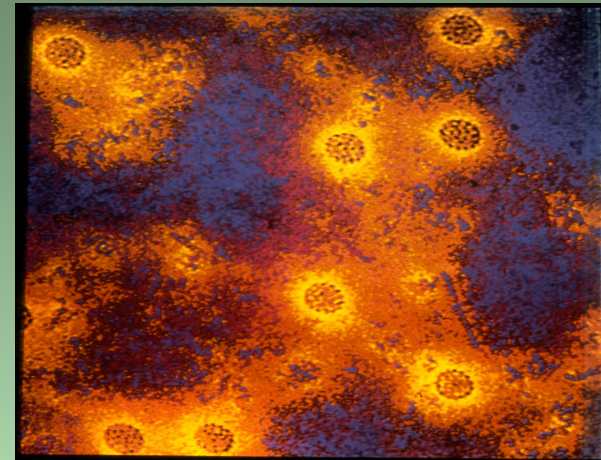
- Pooled HPV high risk positive specimen (genotypes 33,56,73) and low risk genotype 54.
- All participants (100%) detected high risk HPV genotypes in the specimen.

Specimen 8775

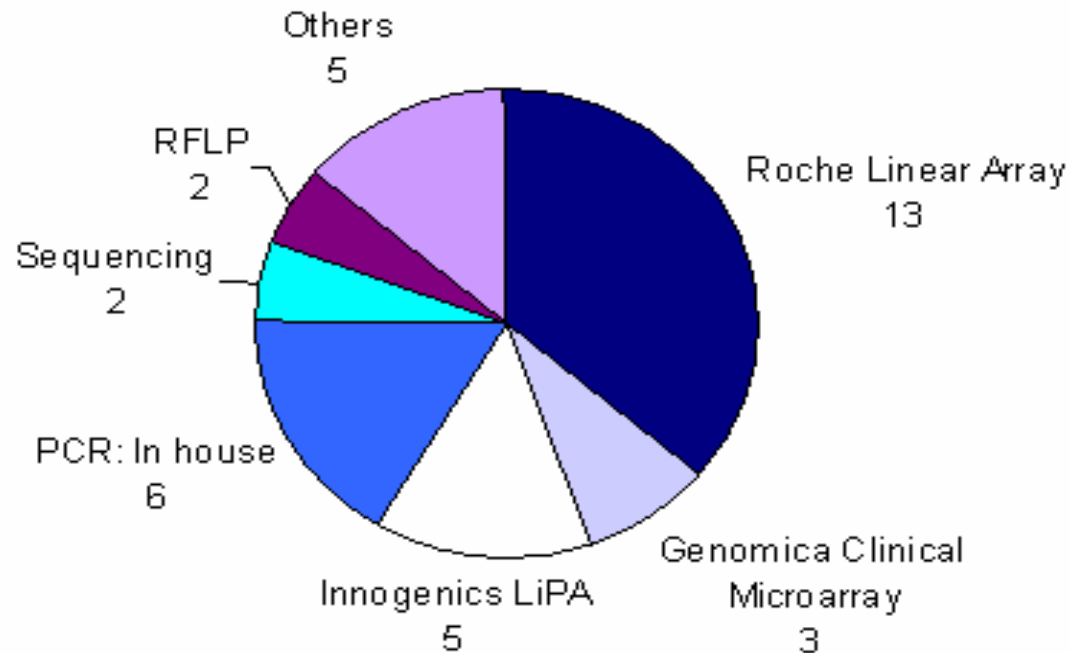
- Negative for high risk genotypes and consisted of preservCyt spiked with MRC-5 cells at a concentration of $0.9 - 1.2 \times 10^5$ cells/mL
- 95% of participants correctly identified this as a HPV high risk negative sample.
- One participant identified genotype 53 using the Roche Linear Array and one reported genotype 66 using the same assay.

Non-Genotyping Methods

- Eleven participants used assays detecting high risk genotypes but did not determine the genotypes present.
- Seven participants used the HR HPV DNA test (Digene)
- Four participants used the Amplicor HPV (Roche)

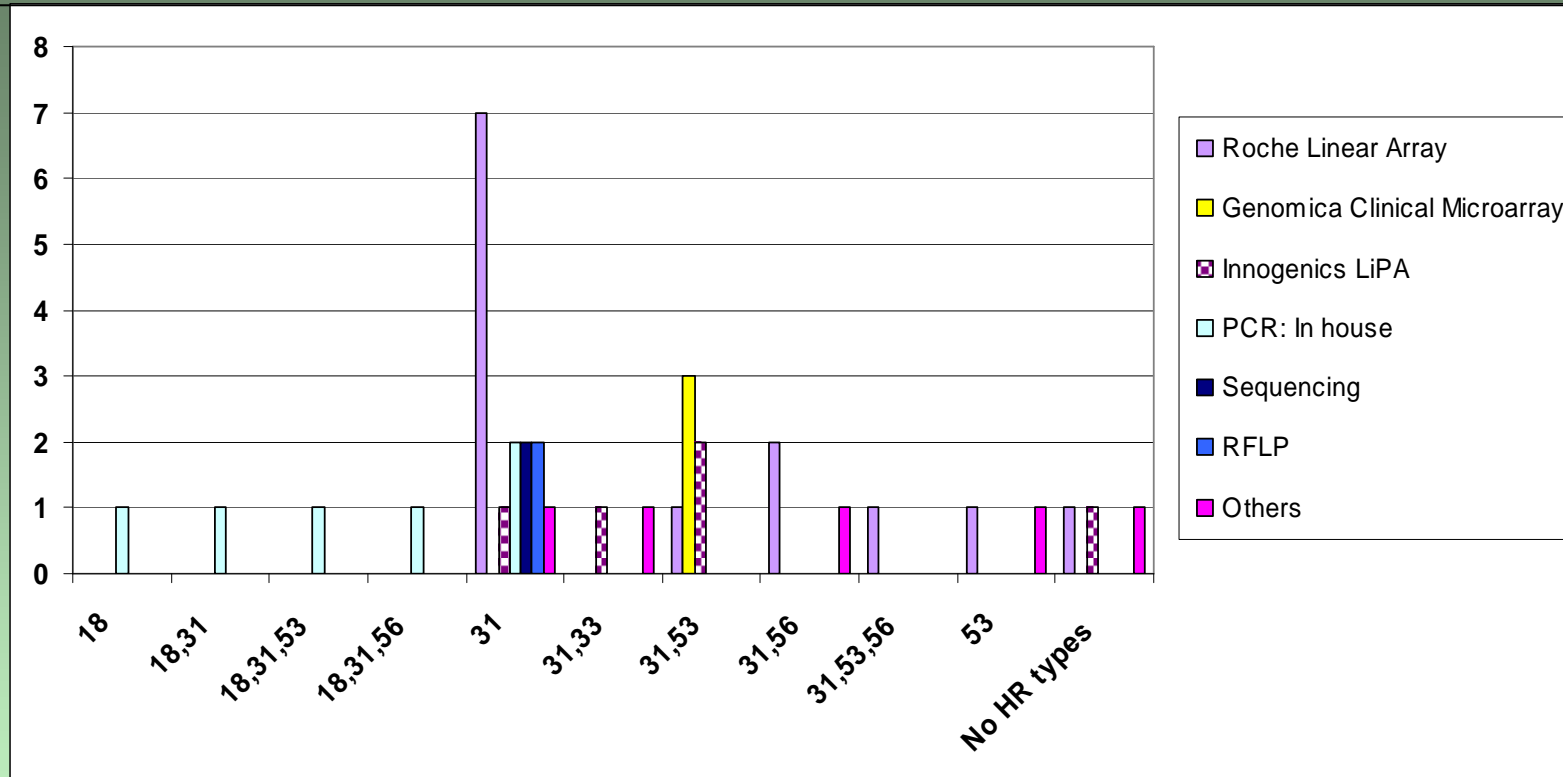


Genotyping Methods



Other methods used included Alphagenic EIA, in-house Microarray and an in-house reverse line blot assay. Two participants did not specify their method.

Specimen 8772: Ref lab result HR 31,53

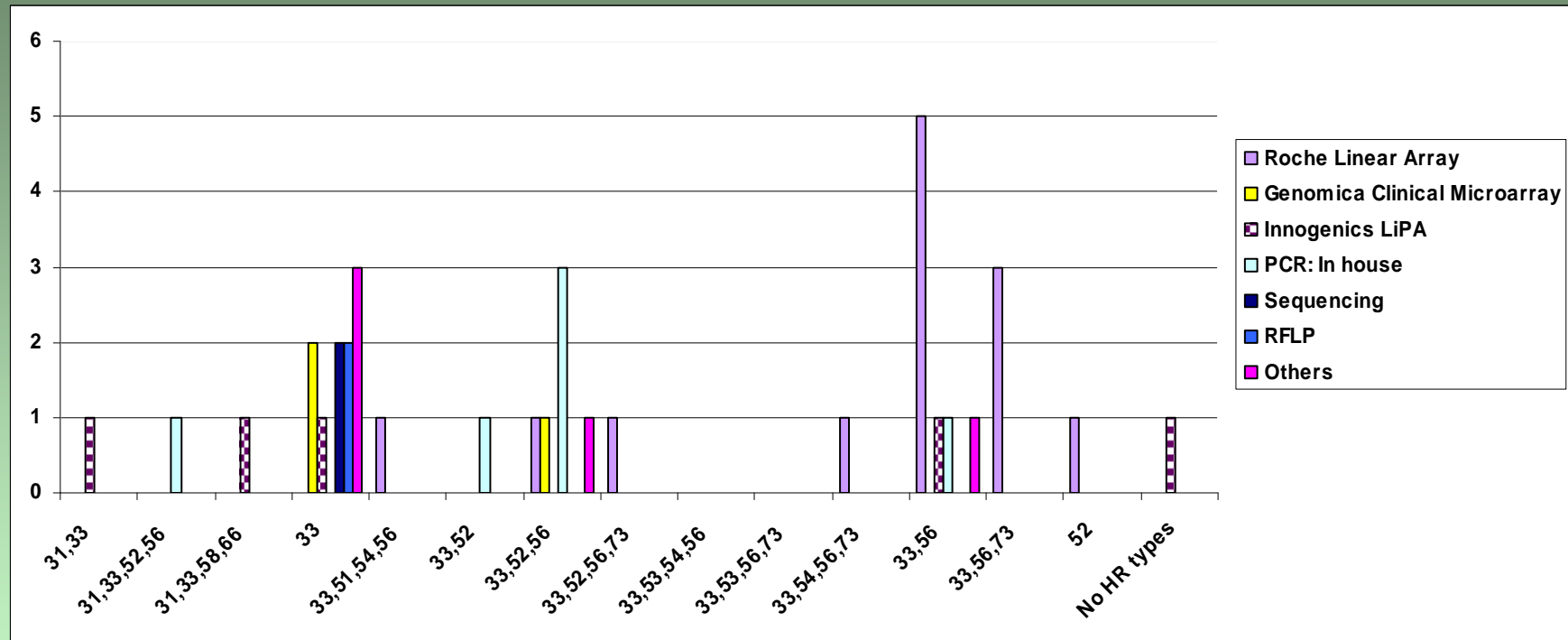


- All three participants using Genomica Clinical Microarray, two labs using Innogenics LiPA and one lab using the Roche Linear Array obtained the same result as the pre-distribution test lab.
- The most commonly detected genotype combination was 31 only.
- Three labs using three different methods failed to detect any HPV genotypes.

Specimen 8772: Ref lab result HR 31,53

- The predominant genotypes correctly reported for this specimen were 31 (29/32 participants) and 53 (10/32 participants).
- Other genotypes reported that were not detected in the specimen by pre-distribution tests were 18 (4 participants) and 56 (5 participants).
- In-house PCR methods detected genotype 18 and genotype 56 was reported by some participants using Roche Linear Array and in-house methods.

Specimen 8774: Ref lab result HR 33,56,73 & LR 54



One participant reported the same results as the pre-distribution test (this was the lab that did the pre-distribution tests !)

Only one participant failed to detect any HR HPV genotypes.

Specimen 8774: Ref lab result HR 33,56,73 & LR 54

- The predominant genotypes correctly reported for this specimen were 33 (33/34 participants), 56 (21 participants), 52 (10 participants) and 73 (2 participants).
- Other genotypes reported were 31 (3 participants), 54 (2 participants), 51, 58 and 66.
- Eight labs detected the genotype combination 33, 56 and 10 labs reported genotype 33 only.

Summary

- Overall the performance was good with 97% of participants reporting correctly on whether they detected HR genotypes or no HPV.
- Genotyping results were variable and combinations of genotypes reported were not associated with any particular method.
- 13 participants using Roche Linear Array reported 6 different genotype combinations for specimen 8772 and 7 for specimen 8774
- Five participants used Innogenics LipA and detected 4 and 5 different genotype combinations in specimens 8772 and 8774 respectively.
- Most participants did not report LR genotypes

Other schemes for introduction in 2009

- MRSA screen
- *Clostridium difficile*
- Genital Pathogens
- Throat infections
- Faecal Pathogens
- Superficial infections
- Special survey: the detection and characterisation of anaerobes

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