The Luminex Platform of the Infections and Cancer Biology Group

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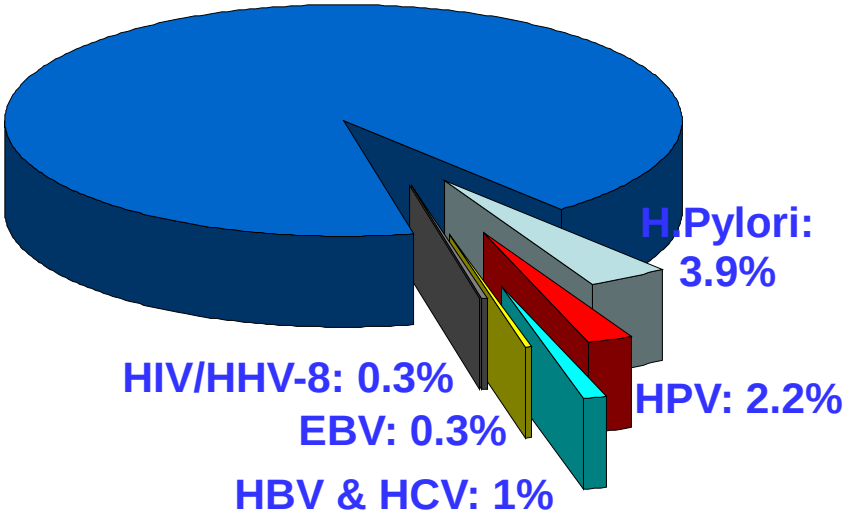
% of cancer cases attributable to infections

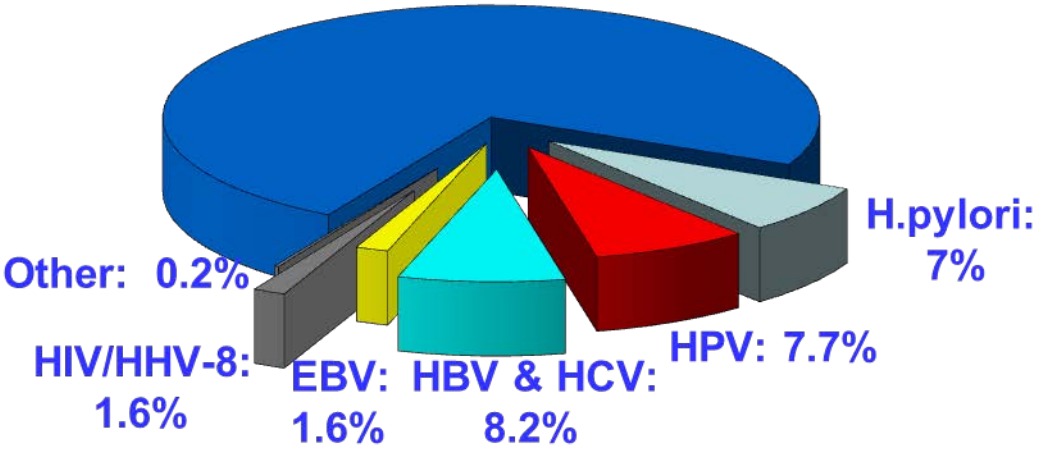
High-resource countries

7,7 % of all cancers



Low-resource countries

27 % of all cancers



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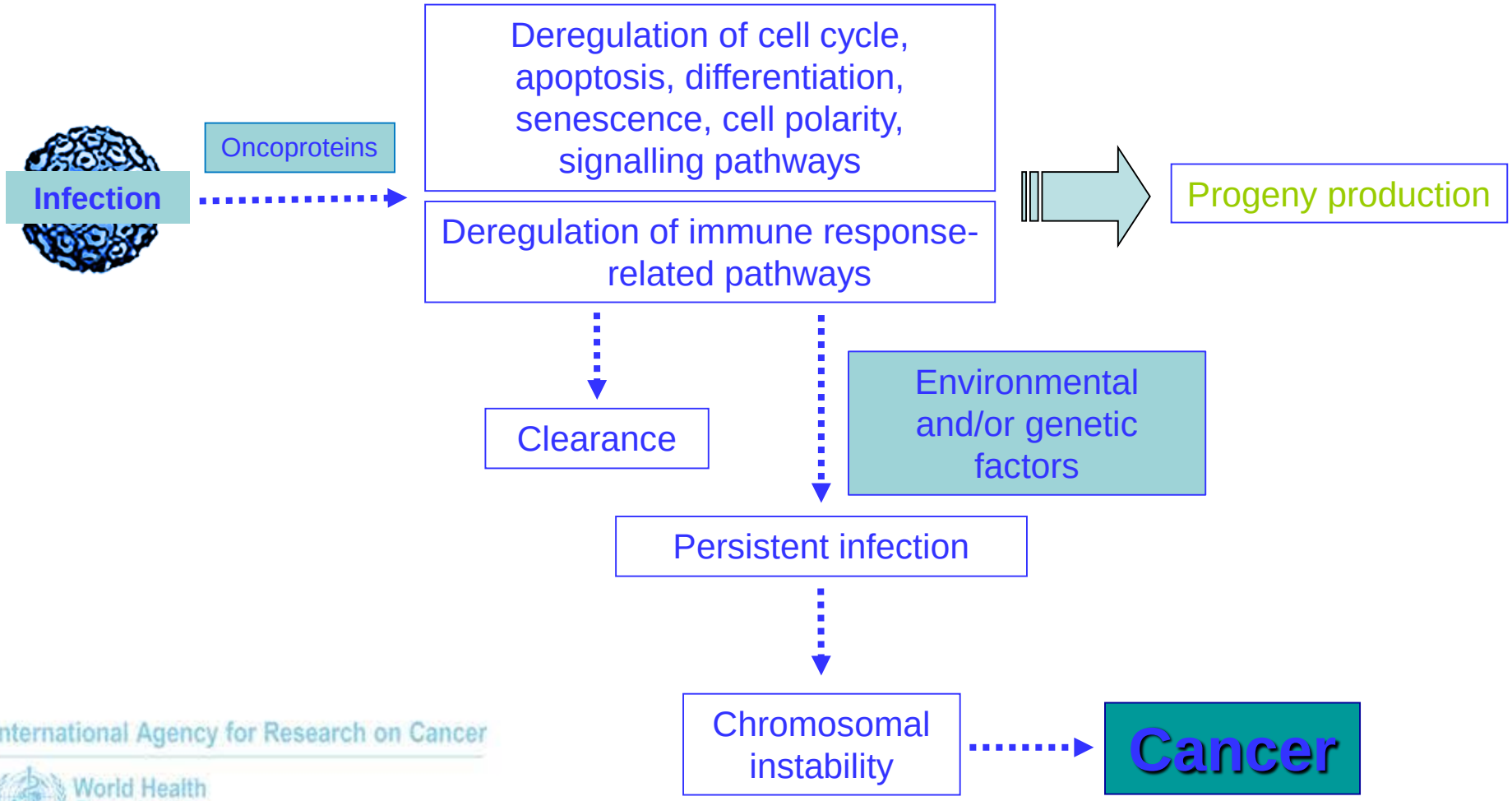
Events associated with the infection of several oncogenic viruses



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Development of novel diagnostic assays

- Rationale

1) Generation of diagnostic cheap tools with high sensitivity, specificity and throughput for broad spectrum of infectious agents to be used in epidemiological studies

2) Possible use of different types of human specimens, including archival material for retrospective studies

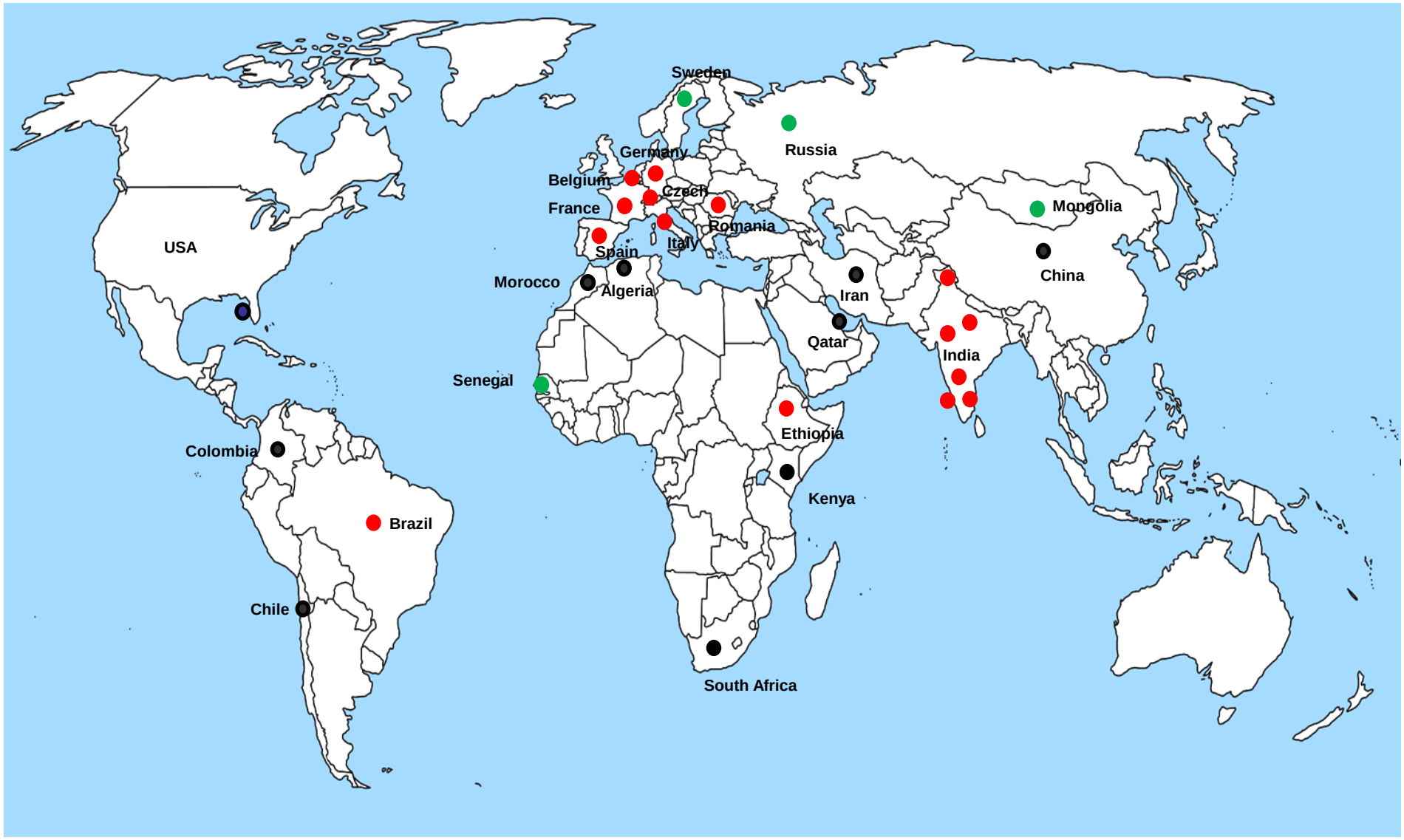
Infectious agents (n=150) included in the IARC Luminex platform

Mucosal high-risk HPV types (n=21)	6, 11,16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68a, 68b, 70, 73 and 82. (+ Chlamydia T.)
Cutaneous gamma HPV (n=52)	4, 48, 50, 60, 65, 88, 95, 101, 103, 108, 109, 112, 116, 119, 121, 123, 126, 127, 128, 129, 130, 131, 132, 133, 134, 148, 149, 156, SD2, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 175, 178, 179, 180, 184, 197, 199, 200, 201, 202
Cutaneous beta HPV types (n=46)	5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 75, 76, 80, 92, 93, 96, 98, 99, 100, 104, 105, 107, 110, 111, 113, 115, 118, 120, 122, 124, 143, 145, 150, 151.152, 159, 174
HPV alpha, mu (n=5)	1, 2, 3, 27, 57
Polyomaviruses (n=12)	BKV, WUV, KIV, MCV, JCV, HPyV6, HpyV7, HpyV9, HpyV10, TSV and SV40, HpyV12
Herpes Viruses (n=8)	HSV1, HSV2, VZ, EBV (1 and 2), CMV, HH6, HH7, HH8,
Other infectious agents (n=6)	Helicobacter Pylori, HBV, HIV, Chlamydia T., Schistosoma (3 different types), Bocavirus

Generation of Luminex platform for bacteria (n=100)

Bacteria that colonize the oral cavity, gut and genital tract: i.e Bacteroidaceae, Bifidobacteriaceae, Campylobacteraceae, Chlamydiaceae, Clostridiaceae, Desulfobacteraceae, Enterobacteriaceae, Enterococcaceae, Eubacteriaceae, Fusobacteriaceae, Helicobacteraceae, Klebsiella pneumonia, Peptostreptococcaceae, Porphyromonadaceae, Propionibacteriaceae, Ruminococcaceae, Streptococcaceae

Some examples of worldwide collaborative studies



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- HNC
- Ano-genital cancers
- Other cancers: i.e. breast, lung, esophagus

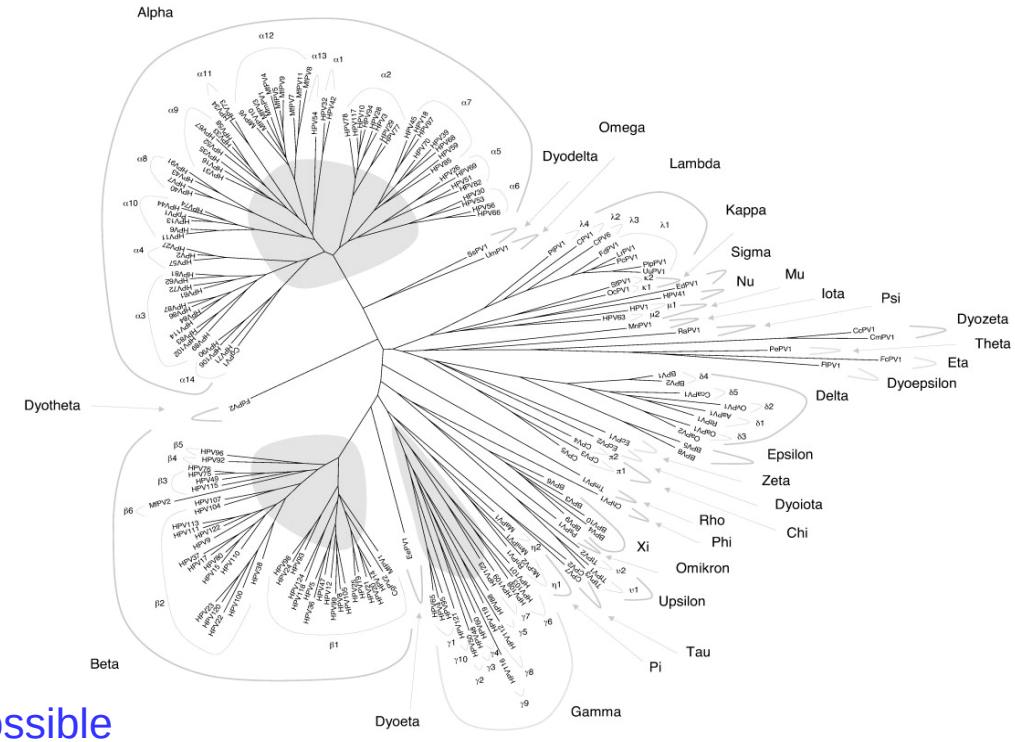
Specimens successfully used so far

(i) Formalin fixed tissues in paraffin blocks
(ii) Frozen tissues
(iii) Exfoliated cervical cells in PBS or fixative (e.g. ThinPrep)
(iv) Urine
(v) Oral brushes, gargles and saliva
(vi) Breast ductal lavages/milk/colostrum
(vii) Cerebrospinal fluids
(viii) Eyebrow hairs
(ix) Skin cells collected using multiple swabbing techniques
(x) Buffy coat
(xi) Stool
(xii) Circulating Tumour DNA

Three small examples

Human papillomaviruses

(i) Mucosal low-risk HPV types, e.g. 6 and 11
(ii) Mucosal high-risk HPV types, e.g. 16 and 18
(iii) Benign cutaneous HPV types, e.g. 2, 3 and 10



Possible association with non-melanoma skin cancers

Cutaneous HPV types, poorly investigated

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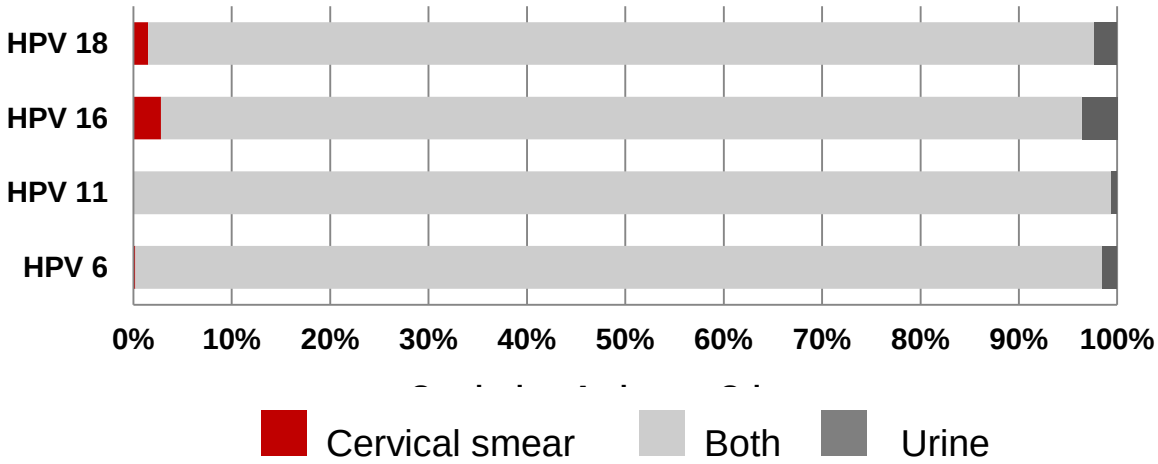
Performance of the Luminex assay in detecting HPV DNA in urine and cervical samples

Concordance in HPV DNA detection in cervical smear and urine



	Accordance (%)	Kappa (95% IC)	McNemar* P value
HPV 18	96,2%	0,546 (0,486-0,605)	**0,3711**
HPV 16	93,6%	0,736 (0,697-0,775)	**0,4927**
HPV 11	99,4%	0,767 (0,734-0,799)	**0,0833**
HPV 6	98,3%	0,815 (0,787-0,843)	0,0196

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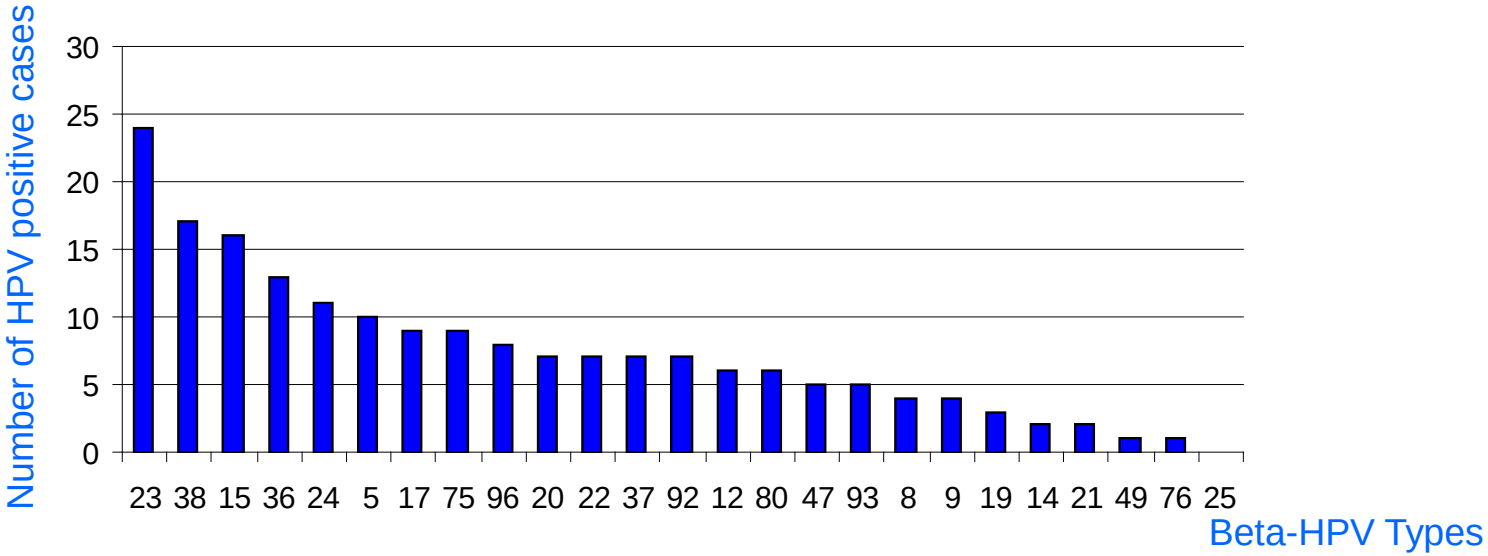
In collaboration with Alba Lucia Combita and colleagues, Bogota, Colombia

HPV flora in oral cavity

- 28% of the samples were positives for High risk mucosal types (HPV16 represented 20%)
- 6% were positives for Low risk mucosal HPV types
- 78% were positives for Cutaneous HPV types from the genus Beta (HPV23, 38, 15 …)



- 13% of the samples contains HPV from the genus Gamma
- Overall, 85% were HPV positives

Oral cavity of healthy individual is colonized by a HPV flora

Circulating tumour DNA

Number cases tested	73		
No tumor	7		
		Tumor HPV status	
CtHPV DNA status	N positives	Positive	Negative
HPV16	18	17	1
HPV18	1	1	
HPV33	2	2	
HPV35	1	1	
HPV58	1	n/a	

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In collaboration with Paul Brennan, IARC

Our interest in child cohorts

Infections

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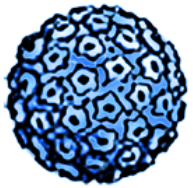

Persistent infection

Environmental and/or genetic factors

?

Cancer

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Infections and environmental factors

E6 and E7 from beta HPV38 cooperate with ultraviolet light in the development of actinic keratosis-like lesions and squamous cell carcinoma in mice.

Viarisio D, Mueller-Decker K, Kloz U, Aengeneyndt B, Kopp-Schneider A, Gröne HJ, Gheit T, Flechtenmacher C, Gissmann L, Tommasino M.
PLoS Pathog. 2011 Jul;7(7):e1002125..

The mycotoxin aflatoxin B1 stimulates Epstein-Barr virus-induced B-cell transformation in in vitro and in vivo experimental models.

Accardi R, Gruffat H, Sirand C, Fusil F, Gheit T, Hernandez-Vargas H, Le Calvez-Kelm F, Traverse-Glehen A, Cosset FL, Manet E, Wild CP, Tommasino M.
Carcinogenesis. 2015 Nov;36(11):1440-51.

Acknowledgements (Main collaborators)

IARC - ICB group

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Uzma Hasan

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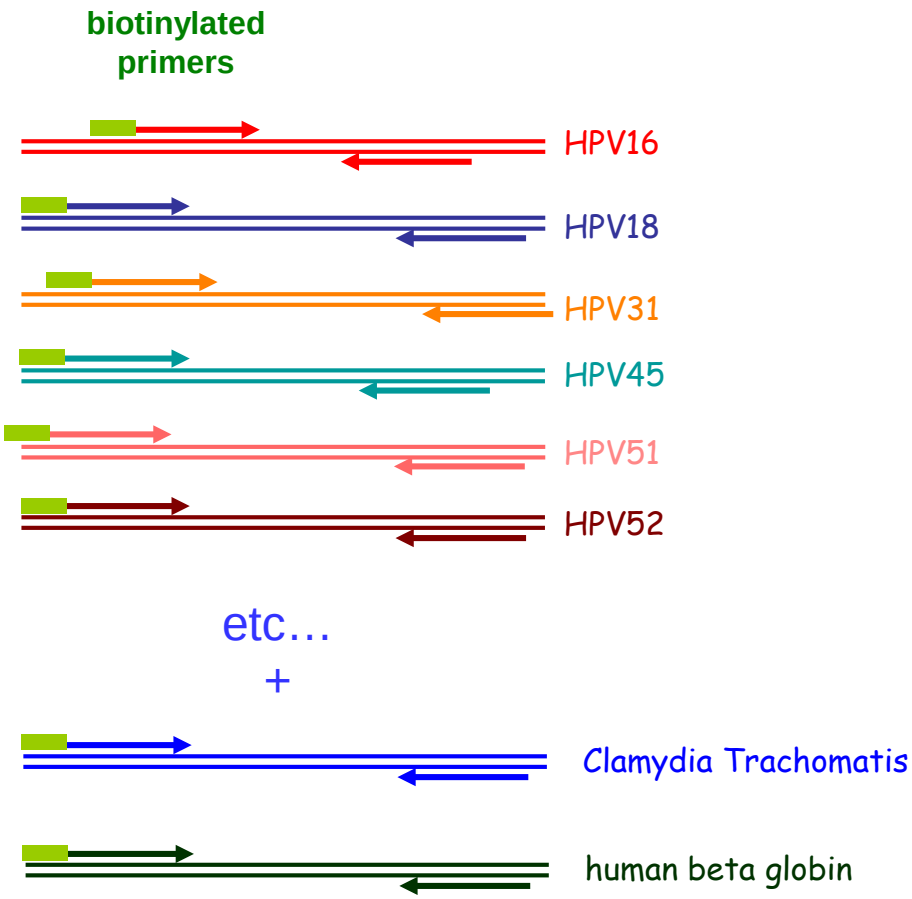
CONTRE LE CANCER LA LIGUE 101 comités

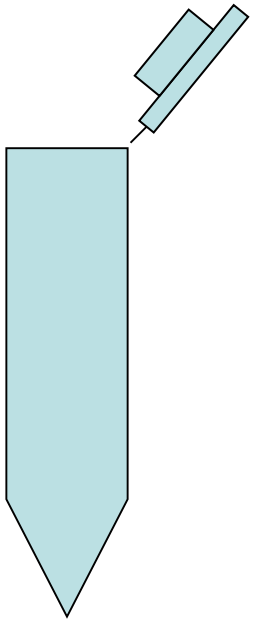
AICR CANCER KNOWS NO BOUNDARIES FORTUNATELY, NEITHER DO WE

SEVENTH FRAMEWORK PROGRAMME

E7 multiplex PCR



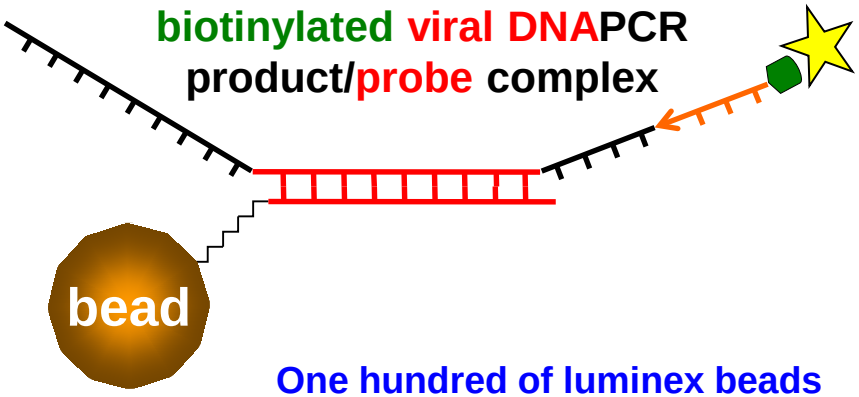
Reaction in a single tube



200bp amplimers

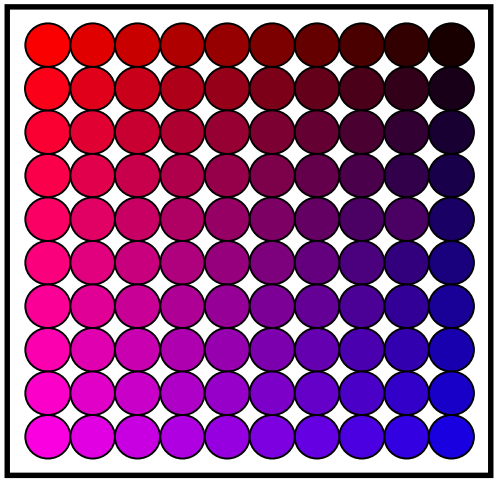
Luminex-based assay

- Multiplex PCR

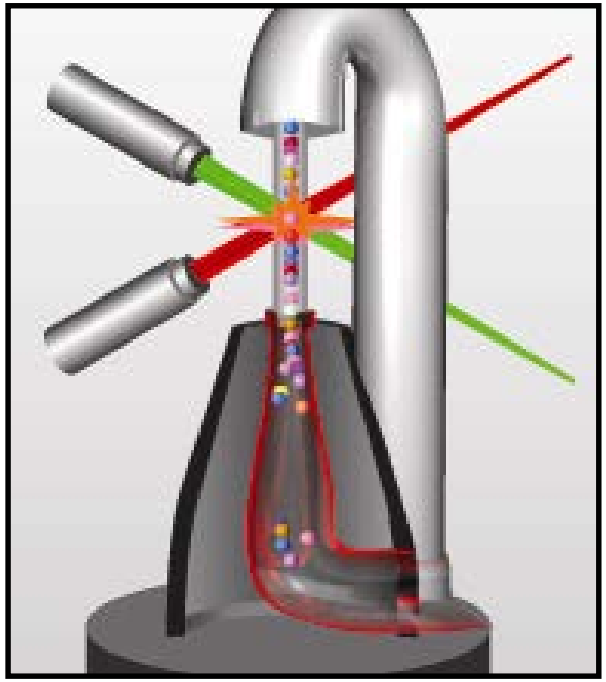


streptavidin-R-phycoerythrin

One hundred of luminex beads with different fluorescent dye are commercially available



Flow cytometer



High specificity of the Luminex-based assay

HPV PCR product	HPV type-specific probe																			
	16_1	16_2	18	26	31	33	35	39	45	51	52	53	56	58	59	66	68	70	73	82
16	**369**	**289**	5	10	4	16	4	13	4	15	4	9	14	9	15	10	8	15	8	6
18	13	2	**701**	11	2	9	3	12	4	12	3	9	7	5	16	6	6	15	8	6
26	14	2	3	**152**	2	10	3	13	4	12	3	8	8	5	15	6	6	23[b]	7	6
31	11	4	4	10	**791**	13	4	12	4	15	4	9	11	8	16	8	8	15	8	7
33	12	3	5	11	3	**152**	3	13	4	16	5	10	12	9	16	9	8	17	8	7
35	12	2	2	11	3	11	**218**	12	4	14	3	9	10	7	15	7	7	15	7	6
39	11	2	4	12	2	10	4	**189**	4	14	3	9	7	4	15	6	6	17	6	6
45	12	2	4	11	2	13	3	14	**1055**	14	5	9	11	8	17	9	7	16	7	7
51	10	3	3	10	2	12	4	12	4	**216**	3	9	9	6	15	8	7	15	6	6
52	11	2	4	11	2	14	4	13	5	13	**114**	8	10	7	16	8	7	16	7	6
53	11	2	4	9	3	12	3	12	4	14	4	**116**	9	7	16	7	8	15	7	6
56	12	3	4	10	2	12	4	12	4	15	5	9	**705**	7	16	9	7	16	7	6
58	11	2	3	9	3	12	3	13	3	14	4	8	9	**218**	16	7	6	15	6	6
59	8	2	3	7	2	10	3	10	4	12	3	7	9	7	**292**	6	6	12	7	5
66	10	2	3	10	3	10	3	12	3	13	4	8	10	7	15	**577**	6	14	6	5
68	12	2	3	11	2	12	4	12	4	12	3	9	9	7	16	8	**332**	16	7	6
70	11	2	3	11	2	12	3	13	3	14	4	9	9	7	18	8	8	**216**	7	6
73	7	2	3	6	2	11	3	8	4	13	3	5	8	7	12	7	6	11	**215**	5
82	11	2	3	8	2	12	3	11	3	13	3	8	8	7	14	7	6	15	6	**227**
None[a]	10	2	4	10	2	10	4	13	4	13	4	8	11	9	13	10	8	13	8	7
Cutoff	17	7	8	17	7	18	8	18	9	20	9	15	15	13	23	13	13	22	13	12

Transfer of the Luminex-based assays to other Institutes

(i) the Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India

(ii) Centro de Investigação Translacional em Oncologia – ICESP Cerqueira César – São Paulo, Brazil

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