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Professional paper

Quality Assurance of Human Papillomavirus Testing

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A B S T R A C T

The External Quality Assurance (EQA) in medical microbiology in the Czech Republic is well organized. It is coordinated by the Accreditation Department of the Centre of Epidemiology and Microbiology (AD-CEM) of the National Institute of Public Health in Prague. Since 1993 when the first samples were sent out the number of programmes and participating laboratories has been rapidly increasing. EQA for Human papillomavirus (HPV) has been available since 2000. As has been shown for other programmes, the EQA for HPV has proved to be useful, helping to improve the accuracy of analyses and contributing to the standardization of methods of HPV DNA testing. EQA for HPV has been well received by routine laboratories, demonstrated by a high number of these institutions voluntarily participating in EQA.

Key words: *external quality assurance (EQA), human papillomavirus (HPV)*

Introduction

Today, most diagnostic laboratories are capable of detecting a wide range of infectious agents. The In Vitro Diagnostic Industry is committed to bringing an even bigger variety of diagnostic assays on the market in the future. Even though the process of certification of *in vitro* diagnostics for infectious agents is obligatory, this is not always a guarantee that the assay is working properly in the end-user's laboratory. Especially for very sensitive molecular diagnostic assays, which often straddle the divide between research and routine, internal and external quality assurance is important. Several institutions worldwide provide panels of reference samples for the most commonly diagnosed infections. For Human papillomaviruses (HPV), the first internationally available panel of samples for quality assurance was offered by Instand (WHO Collaborating Centre for Quality Assurance and Standardization in Laboratory Medicine)[1] in 2004.

Long Tradition

The system of External Quality Assessment (EQA) in the Czech Republic was introduced in 1993. The first programme focused on sera for detection of the hepatitis B virus and strains of bacteria for identification. From 1993 till 2002 the number of provided surveys increased from 2 to 34 and the number of participating laboratories increased from 79 to 440 (Figures 1 and 2). In order to provide technical support for this programme, a coordinating centre for EQA in medical microbiology was established (the Accreditation Department of the Centre of Epidemiology and Microbiology (AD-CEM), National Institute of Public Health). Since then, AD-CEM has devel-

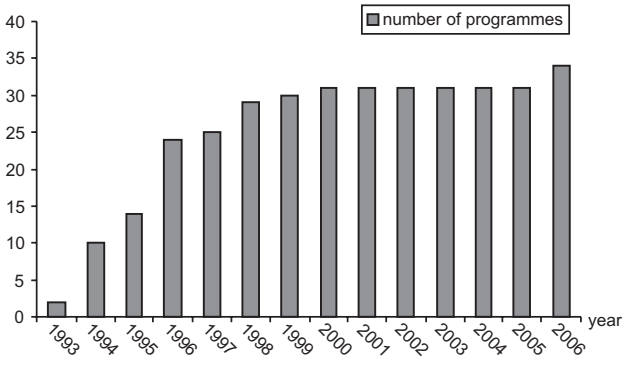

Fig. 1. Number of programmes provided by the Accreditation Department of the Centre of Epidemiology and Microbiology (AD-CEM) from 1993–2006[2].

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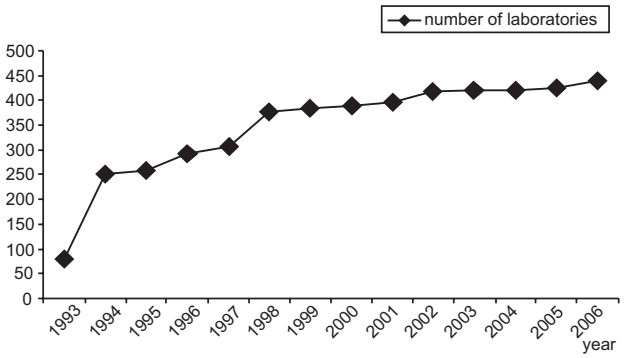

Fig. 2. Number of laboratories registered in the External Quality Assurance (EQA) in the Czech Republic from 1993–2006[2].

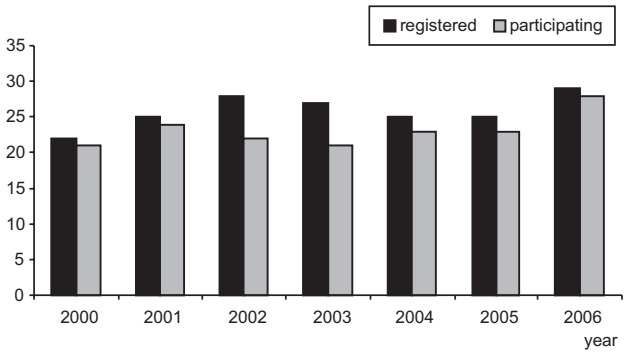

Fig. 3. Number of laboratories registered and participating in EQA for Human papillomavirus (HPV) testing in the Czech Republic from 2000–2006[2,3].

oped a wide network of national reference laboratories and some other highly specialized centres, which prepare samples for EQA[2].

AD-CEM distributes the coded samples together with all required protocols containing detailed instructions. Each programme has an individual deadline for receiving the results. AD-CEM sends the protocols with the results to the contractual laboratory for evaluation. Each participating laboratory in this system has a unique identification code and the evaluation of results is therefore anonymous. The certificate of participation is issued to all participants who registered for the programme in the particular year and those who successfully passed the EQA also obtain the Certificate of proper diagnostics.

After ten years of its existence, AD-CEM has achieved some very positive outcomes. First of all, the accuracy of analyses has increased and methods have become better standardized. In addition, significant variations have been detected between different diagnostic sets; as a result, those sets that have yielded unsatisfactory results have been eliminated.

Participation in the system of EQA is not compulsory, but most of the routine laboratories voluntarily take part since it allows them to compare the performance of a wide variety of detection techniques and diagnostic sets with many others laboratories. All results are published in the journal, »Zprávy CEM« (News of CEM), issued by the Centre of Epidemiology and Microbiology, National Institute of Public Health as well as on the websites of some of the contractual laboratories.

External Quality Assurance for HPV

In 1998, the National Reference Laboratory (NRL) for Papillomaviruses was established by the National Public Health Authority on the recommendation of the Working Group for Virology of the Society for Clinical Microbiology of the Czech Medical Association JEP to improve the interpretation of results and disseminate information concerning the clinical usefulness of HPV DNA detection among specialists.

Since 2000, 5 EQA sample series have been distributed annually to each of the participating laboratories (Figure 3). Since 2003, EQA has also been provided to laboratories using PCR-based diagnostic kits. EQA is carried out depending on the diagnostic kit used (i.e. depending on whether the kit detects both low risk [LR] and high risk [HR] HPV types or HR HPV types only). If the kit used detects both LR and HR HPV types, the participating laboratory is awarded a score of 2 points for each correct result and loses 1 point when failing to detect either LR or HR HPV types. When an incorrect result is reported for a sample with a borderline amount of virus for a detection limit of the given kit (provided the test is recommended for diagnostic use by the NRL), NRL requires correct interpretation of any equivocal result. If possible, NRL informs the participating laboratory about the mechanism likely to be responsible for the error (e.g. low sensitivity of the method, defective kit batch, improper performance of the test, sample contamination, etc.).

The samples for EQA are prepared to simulate the cervical smear samples taken to a transport medium. At the beginning of EQA, the Specimen Transport Medium (STM, Digene) was used. Later, when PCR-based commercially available sets appeared, samples prepared in the PreservCyt medium (Cytyc) for PCR-based methods were used. Recently, we finished a study whose results show the possibility of using STM medium for both non-amplification and amplification-based methods. In each sample, a background of 100,000 HPV negative cells is included. Each series contains an HPV negative, LR HPV positive and HR HPV positive sample and also a sample positive for both LR and HR HPV. Both HPV positive cell lines and cloned DNA is used for sample preparation. Different amounts of HPV positive cells and/or cloned DNA are used to simulate different viral load and verify the sensitivity of methods used in laboratories. To illustrate, we include a table showing the results of participating laboratories in 2001 and 2006 (Table 1 and 2)[3].

At the beginning of EQA for HPV, most of the laboratories used Hybrid Capture Tube test (HCT, Digene), which has been shown to have a limited sensitivity for

TABLE 1
RESULTS OF EQA FOR HPV IN 20013

Test	Laboratory number.	Sample No.1		Sample No.2		Sample No.3		Sample No.4		Sample No.5		Rating
		LR RLU/CO	HR RLU/CO	LR RLU/CO	HR RLU/CO	LR RLU/CO	HR RLU/CO	LR RLU/CO	HR RLU/CO	LR RLU/CO	HR RLU/CO	
HCT Digene	26	0.25	0.24	0.33*	0.25	0.25	0.26*	0.73*	0.42*	0.26	0.26*	2.0
HCT Digene	118	0.19	0.25	0.48*	0.26	0.25	0.58*	1.44	1.03	0.34	2.75	6.0
HCT Digene	211	0.58	0.26	0.72*	0.30	0.62	0.35*	1.38	0.64*	0.54	0.31*	2.0
HCT Digene	28	0.23	0.24	0.32*	0.20	0.22	0.34*	0.75*	0.40*	0.20	0.24*	0.0
HCT Digene	371	0.28	0.49	0.39*	0.32	0.25	0.35*	0.77*	0.63*	0.25	1.04	4.0
HCT Digene	406	0.89	1.28**	ND	ND	0.67	2.01	ND	ND	3.33**	0.72*	0.0±4.0
HCT Digene	18	3.78**	3.6**	0.53*	0.99	0.31	0.68*	3.84	1.41	0.29	0.26*	0.0
HCT Digene	192	0.37	0.30	0.45*	0.38	0.37	0.38*	1.07	0.64*	0.43	0.43*	2.0
HC2 Digene	165	0.14	0.17	1.18	0.17	0.19	0.87*	7.02	2.60	0.16	16.89	8–9.0
HC2 Digene	373	0.93	0.19	2.44	0.20	1.14**	1.47	1.81	1.82	0.82	17.93	8.0
HC2 Digene	156	0.18	0.14	1.04	0.15	0.16	1.30	3.42	2.21	0.18	18.90	10.0
HC2 Digene	115	0.20	0.50	1.41	0.16	0.29	1.58	6.30	2.36	0.22	16.78	10.0
HC2 Digene	585	0.54	1.34**	1.88	2.00**	2.12**	2.92	4.71	2.21	5.67**	22.21	2.0
HC2 Digene	584	0.20	0.21	1.42	0.20	0.36	1.38	6.79	3.16	0.20	18.99	10.0
HC2 Digene	13	0.34	0.37	1.75	0.40	0.27	1.38	10.51	2.61	0.26	18.84	10.0
HC2 Digene	344	0.24	0.21	1.04	0.50	0.23	1.20	4.16	1.70	0.38	11.89	10.0
HC2 Digene	405	0.59	0.24	1.28	0.47	0.23	1.38	4.53	2.05	0.51	15.77	10.0
HC2 Digene	31	0.34	0.25	1.69	0.36	0.31	2.43	6.36	2.83	0.45	34.82	10.0
HC2 Digene	315	0.40	0.70	1.12	0.40	0.30	1.20	4.50	2.40	0.50	15.20	10.0
HC2 Digene	147	0.56	0.47	1.72	0.28	0.55	1.38	6.67	2.68	0.46	18.40	10.0
HC2 Digene	407	0.57	0.52	1.14	0.77	0.46	1.60	3.86	2.29	0.45	13.97	10.0
HC2 Digene	11	0.15	0.26	1.02	0.20	0.13	1.13	3.51	1.63	0.19	12.85	10.0
HC2 Digene	230	0.23	0.19	1.06	0.18	0.20	1.22	4.09	1.82	0.22	15.56	10.0
HC2 Digene	471	0.16	0.14	1.43	0.15	0.17	1.36	5.67	2.70	0.18	18.75	10.0
HC2 Digene	NRL PV	0.35	0.33	1.67	0.23	0.39	1.73	7.27	2.64	0.35	18.63	10.0

EQA – External Quality Assurance, HPV – Human Papillomavirus, * – error caused by the low sensitivity of the detection method, ** – probable contamination of samples, HCT – Hybrid Capture 1 HPV test (Digene), HC2 – Hybrid Capture 2 HPV test (Digene), LR – low risk, HR – high risk, RLU/CO – relative light units/cut-off, ND – not done, NRL PV – National reference laboratory for papillomaviruses. Available at: http://www.papillomavirus.cz/eng/activities_eqa.html.

the detection of CIN2+ lesions. Therefore, in the EQA in 2000 we set the cut-off line just above the detection level for HCT. We recommended that all laboratories plan to switch to HC2 (Digene) (primarily because at that time no other commercial sets were available on the Czech market). The reaction was quite positive and as you can see from Table 3, the spectrum of diagnostic sets used in routine laboratories changed. In 2000, 50% (11/21) of laboratories in EQA used HCT, but this number decreased to 3 out of 22 in 2002 and just 1 in 23 laboratories in 2004. 2004 was the first year when 3 laboratories used PCR-based HPV DNA tests (HPV INNO-LiPA Innogenetics, Amplicor HPV Test Roche, DNA PCR Test Gentech).

Discussion

From the onset of EQA, we regularly detected mistakes in using even such robust sets as HC2. However, over the years, the performance of laboratories in EQA improved and in the last two years we have had no mistakes reported from the laboratories using HC2 or HC2 HR sets. In 2006, we had 6 laboratories using commercially available PCR-based sets. The results of EQA from laboratories which use PCR-based sets show bigger heterogeneity and confirm the need for participation in the EQA (Table 2)[3].

In the absence of an internationally available programme for EQA for HPV, we exchanged the samples with the Laboratory of Clinical and Epidemiological Virology, Rega Institute for Medical Research, University of Leuven, Belgium in 2002. In 2004 Instand (WHO Collaborating Centre for Quality Assurance and Standardization in Laboratory Medicine)[1] for the first time offered EQA for HPV and since then, NRL has been successfully participating in this programme. In 2004, Instand prepared samples which had a limited amount of HPV DNA (below the cut-off for HC2 Digene). Since we used different

TABLE 2
RESULTS OF EQA FOR HPV TESTING IN 2006[3]

Test	Laboratory number	Sample No.1		Sample No.2		Sample No.3		Sample No.4		Sample No.5		Rating
		LR RLU/CO	HR RLU/CO	LR RLU/CO	HR RLU/CO	LR RLU/CO	HR RLU/CO	LR RLU/CO	HR RLU/CO	LR RLU/CO	HR RLU/CO	
HC2 Digene	11	24.43	0.15	0.11	3.79	20.76	0.13	0.11	0.11	0.12	6.51	10.0
HC2 Digene	13	24.60	0.25	0.32	4.41	24.18	0.23	0.19	0.15	0.15	6.43	10.0
HC2 Digene	31	29.23	0.15	0.10	4.61	30.53	0.18	0.11	0.13	0.12	7.83	10.0
HC2 Digene	79	30.46	0.12	0.08	4.76	30.74	0.13	0.11	0.09	0.08	7.63	10.0
HC2 Digene	115	27.31	0.16	0.16	4.09	23.01	0.16	0.14	0.15	0.12	7.15	10.0
HC2 Digene	118	28.83	0.15	0.11	4.54	27.00	0.15	0.10	0.09	0.10	6.47	10.0
HC2 Digene	156	29.49	0.16	0.11	4.43	22.56	0.36	0.13	0.13	0.18	5.53	10.0
HC2 Digene	165	29.30	0.26	0.13	4.98	29.80	0.21	0.15	0.20	0.14	6.94	10.0
HC2 Digene	192	28.71	0.10	0.09	4.51	25.35	0.08	0.10	0.08	0.10	6.10	10.0
HC2 Digene	230	18.02	0.79	0.46	2.47	18.91	0.75	0.55	0.46	0.43	4.74	10.0
HC2 Digene	315	26.70	0.10	0.10	3.60	27.90	0.20	0.10	0.10	0.10	5.60	10.0
HC2 Digene	325	30.36	0.19	0.12	4.54	31.74	0.18	0.14	0.14	0.13	8.27	10.0
HC2 Digene	344	27.37	0.35	0.11	3.56	29.95	0.18	0.21	0.15	0.47	6.33	10.0
HC2 Digene	354	20.68	0.10	0.08	3.51	19.98	0.10	0.12	0.06	0.10	2.85	10.0
HC2 Digene	369	18.22	0.54	0.63	2.95	16.47	0.49	0.38	0.54	0.38	4.45	10.0
HC2 Digene	371	28.88	0.16	0.07	4.22	26.02	0.25	0.10	0.11	0.12	6.73	10.0
HC2 Digene	373	3.16	0.16	0.15	4.40	26.60	0.17	0.16	0.17	0.16	6.69	10.0
HC2 Digene	407	25.00	ND	ND	ND	31.00	ND	ND	ND	ND	6.00	10.0
HC2 Digene	585	27.46	0.26	0.45	4.38	23.70	0.27	0.23	0.24	0.18	7.45	10.0
HC2 Digene	734	29.37	0.16	0.09	4.85	27.72	0.14	0.09	0.13	0.10	7.17	10.0
HC2 HR Digene	584	–	0.19	–	4.45	–	0.14	–	0.12	–	7.38	10.0
HC2 HR Digene	736	–	0.13	–	5.15	–	0.14	–	0.21	–	8.21	10.0
HC2 Digene	NRL PV1	28.50	0.13	0.10	4.65	29.50	0.12	0.10	0.13	0.15	7.59	10.0
HC2 Digene	NRL PV2	26.70	0.16	0.11	4.51	30.18	0.14	0.15	0.10	0.11	7.41	10.0

PCR test	Laboratory No.	Sample No.1		Sample No.2		Sample No.3		Sample No.4		Sample No.5		Rating
		LR HPV	HR HPV	LR HPV	HR HPV	LR HPV	HR HPV	LR HPV	HR HPV	LR HPV	HR HPV	
HPV INNO-LiPA Innogenetics	211	neg**	HPV 31**	neg	HPV 16	HPV 6, 11**	HPV 18, 51, 52, 58, 66**	neg	neg	neg	HPV 16, 35, 45**	6.0
HPV INNO-LiPA Innogenetics	44	HPV 6	neg*	neg	HPV 16	HPV 6	neg	neg	neg	neg	HPV 16, 39	10.0
HPV INNO-LiPA Innogenetics	734	HPV 6	neg*	neg	HPV 16	HPV 6	neg	neg	neg	neg	HPV 16, 39	10.0
DNA PCR Test Gentech	16	poz	poz	neg	neg**	poz	neg	neg	neg	neg	poz	9.0
Amplicor HPV Test Roche	365	–	poz	–	poz	–	neg	–	neg	–	poz	10.0
Amplicor HPV Test Roche	716	–	neg*	–	poz	–	neg	–	neg	–	poz	10.0
Amplicor HPV Test Roche	NRL PV	–	poz	–	poz	–	neg	–	neg	–	poz	10.0
"in house" PCR GP5+/6+bio	NRL PV	HPV 6	HPV 33	0	HPV 16	HPV 6	0	0	0	0	HPV 16, 39	10.0

EQA – External Quality Assurance, HPV – Human Papillomavirus, * – borderline concentration of HPV DNA, ** – error, LR – low risk, HR – high risk, RLU/CO – relative light units/cut-off, ND – not done, NRL PV – National reference laboratory for papillomaviruses. Available at: http://www.papillomavirus.cz/eng/activities_eqa.html

TABLE 3
NUMBER OF LABORATORIES PARTICIPATING IN EQA FOR HPV BY TESTING YEAR AND THE DIAGNOSTIC SET

Year	Number of laboratories registered for the programme	Number of laboratories participating in the programme	Detection method					
			HCT Digene	HC2 Digene	HC2 HR Digene	HPV INNO-LiPA Innogenetics	Amplicor HPV Test Roche	DNA PCR Test Gentech
2000	22	21	11	10	–	–	–	–
2001	25	24	8	16	–	–	–	–
2002	28	22	3	19	–	–	–	–
2003	27	21	2	18	–	1	–	–
2004	25	23	1	19	–	2	–	1
2005	25	23	–	19	–	2	1	1
2006	29	28	–	20	2	3	2	1

EQA – External Quality Assurance, HPV – Human Papillomavirus, HCT – Hybrid Capture 1 HPV test (Digene), HC2 – Hybrid Capture 2 HPV test (Digene), HR – high risk, HPV INNO-LiPA – Line blot assay (Innogenetics), Amplicor HPV Test – Polymerase chain reaction HPV based test (Roche), DNA PCR Test – Polymerase chain reaction HPV based test (Gentech)

methods for assessment of EQA in our laboratory, this discrepancy was obvious and we sent our comments to the producers of EQA, which resulted in the change of the HPV DNA amount in the ECQ in 2005.

Conclusion

EQA in medical microbiology in the Czech Republic is well organized. It is coordinated by AD-CEM of the National Institute of Public Health in Prague. First samples were sent out in 1993. EQA for HPV has been available since 2000 and in 2006, 29 laboratories participated in this programme. As has been shown for other programmes, the EQA for HPV has proved to be useful, helping to improve the accuracy of analyses and contributing to the standardization of methods of HPV DNA testing. EQA for HPV has been well received by routine laboratories, demonstrated by a high number of these institutions voluntarily participating in EQA.

Acknowledgements

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OSIGURANJE KVALITETE TESTOVA NA HUMANI PAPILOMAVIRUS (HPV)

SAŽETAK

Vanjska provjera kvalitete (EQA, od engl. *External Quality Assurance*) je dobro organizirana u medicinskoj mikrobiologiji u Republici Češkoj. Koordinirana je od Odjela za akreditaciju centra za epidemiologiju i mikrobiologiju (AD-CEM, od engl. *Accreditation Department of the Centre of Epidemiology and Microbiology*) Nacionalnog instituta javnog zdravstva u Pragu. Od 1993. godine, kada su prvi uzorci poslani vani, broj programa i uključenih laboratorija je naglo narastao. EQA je za humani papilomavirus (HPV) bila u upotrebi od 2000. godine. Kako je bilo prikazano za druge programe, EQA se pokazala korisnom za HPV, pomažući tako povećanju točnosti analiza i doprinoseći standardizaciji metoda HPV-DNK-testova. EQA je dobro prihvaćena za HPV od strane laboratorija koji vrše rutinske analize, na što ukazuje dobrovoljno sudjelovanje velikog broja ovih institucija u EQA.