



UK Clinical Virology Network

Performance of in-house real-time PCR assays for the detection of adenovirus

Graeme O'May

Scientific Co-ordinator, UK Clinical Virology Network¹
West of Scotland Specialist Virology Centre

Katrina Barlow, Keith Perry

Microbiological Diagnostics Assessment Service
Evaluations and Standards Laboratory²

¹Study co-ordinator, report writer
West of Scotland Specialist Virology Centre
1053 Great Western Road
Glasgow
G12 0ZA

²Study investigator, report writer
Health Protection Agency - Centre for Infections
61 Colindale Avenue
London
NW9 5EQ

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Summary

Laboratories across the UK are using a range of different in-house assays for the detection of viral targets. A small-scale assessment of nine such assays in use for the detection for adenovirus infections indicated that the majority of these assays perform at an equivalent level of sensitivity.

Introduction

Laboratories across the UK are using a range of different in-house assays for the detection of viral targets, but no comparative study on their performance has been performed. To address this, the Clinical Virology Network (CVN) set up an assessment of these in-house PCR assays, and in collaboration with the Health Protection Agency Microbial Evaluations Centre (HPA-MEC) produced a panel of specimens to gain some insight into how the different assays compare to enable adoption of best practice.

Materials and Methods

Laboratories were invited via the CVN to submit SOPs of in-house real-time assays in regular diagnostic use to the study. A summary of these is included in Appendix 1.

A panel of 20µl aliquots of extracted DNA generated from both clinical and tissue-culture grown material was prepared at the West of Scotland Specialist Virology Centre (WOSSVC). This incorporated two different extraction methods to avoid any bias associated with this process: Qiagen BioRobot and BioMérieux easyMAG. The panel composition is given in Table 1. Two nucleic acid extraction methods were used to act as a control for any variation in extract quality caused by sample extraction methods. The negative clinical sample (a citrated blood) was confirmed as such at WOSSVC by triplicate realtime PCR assays, all of which were negative.

Table 1: Specimen panel composition (for adenovirus)

Sample type	Extraction method	No. of samples	Details
Virus-positive tissue culture fluid	Qiagen BioRobot 9604	9	Extract diluted to limit of originating lab's assay ± four fivefold dilution steps
Virus-positive tissue culture fluid	BioMérieux easyMAG	9	Extract diluted to limit of originating lab's assay ± four fivefold dilution steps
Clinical sample (swab)	Qiagen BioRobot 9604	7	Clinical material from six known adenovirus-positive and one negative specimens
TOTAL		25	

Appropriate dilutions of the extracted material (prepared using nuclease-free water) were established by the WOSSVC using their adenovirus realtime PCR diagnostic assay

SOP. The panel was distributed on dry ice to the participating laboratories, to be stored at -70°C and tested within 10 working days of receipt.

Results

Six laboratories submitted SOPs for inclusion in the study; all returned results. Respondents provided information on C_t (cycle at which the signal crosses the threshold signifying positivity), run validation and interpretation of results. One laboratory (Bart's) had the panel mistakenly delivered to them at room temperature.

The results of three samples (ADV22, 23 & 24) were removed due to difficulties confirming their positivity status. These were confirmed—by triplicate assays—as adenovirus DNA positive at the time the panels were put together. When data from participating laboratories was received, however, all but two reported these samples as adenovirus DNA negative. Those labs which did detect DNA reported only low levels in one or two of these samples. When the samples held in reserve at WOSSVC were re-assayed, all were found to be negative, again using triplicate determinations. Therefore, it seems that something happened to these samples between the initial testing and when I despatched them to participating laboratories.

Table 2 details the number of positive clinical specimen and serial dilution tests recorded by each participating laboratory. Results from Glasgow were obtained by the diagnostic staff of that laboratory, completely independently of the Scientific Co-ordinator.

Table 2. Number of positive specimens from each laboratory

Laboratory	ADV-positive dilutions ($n=15$)	ADV-positive clinical specimens ($n=7$)
Aberdeen	9	7
Barts'	6	6
Birmingham	8	5
Glasgow	8	6
Leeds	8	5
Newcastle	8	7
Mean	7.83	6.0

Birmingham provided quantitative results. These have not been shown here because no comparative data are available.

Several laboratories (Newcastle, Birmingham, Bart's) performed duplicate assays with the material provided. This resulted in some cases where a sample was reported as negative when DNA was detected at a low level in one of the replicates. Newcastle SVU provided a full explanation of their policy with regard to borderline results stating that 'all samples where crossing points are >41 cycles are repeated, if result fails to repeat then the report issued depends on the clinical situation following discussion by the virology medical staff with the appropriate clinician'.

A graphical representation of results—as C_t values—is given in Figure 1. Only one laboratory distinguished between 'positive' and 'low positive' (Glasgow). A breakdown of positive and negative results reported for the clinical samples is shown in Figure 2. Most

laboratories had similar levels of detection; Only Aberdeen was able to detect DNA in sample ADV31, whilst only Newcastle would report ADV21 as negative, although DNA was detected in this sample.

Two laboratories (Aberdeen and Newcastle) detected adenovirus DNA in the clinical sample confirmed as negative at WOSSVC. Whether this is a genuine detection which was missed by WOSSVC during panel preparation or simply a false positive is unknown; although the fact that DNA was detected in this sample by two laboratories (and by duplicate determinations at Newcastle) lends weight to the former supposition. Further investigation is required to fully elucidate the facts.

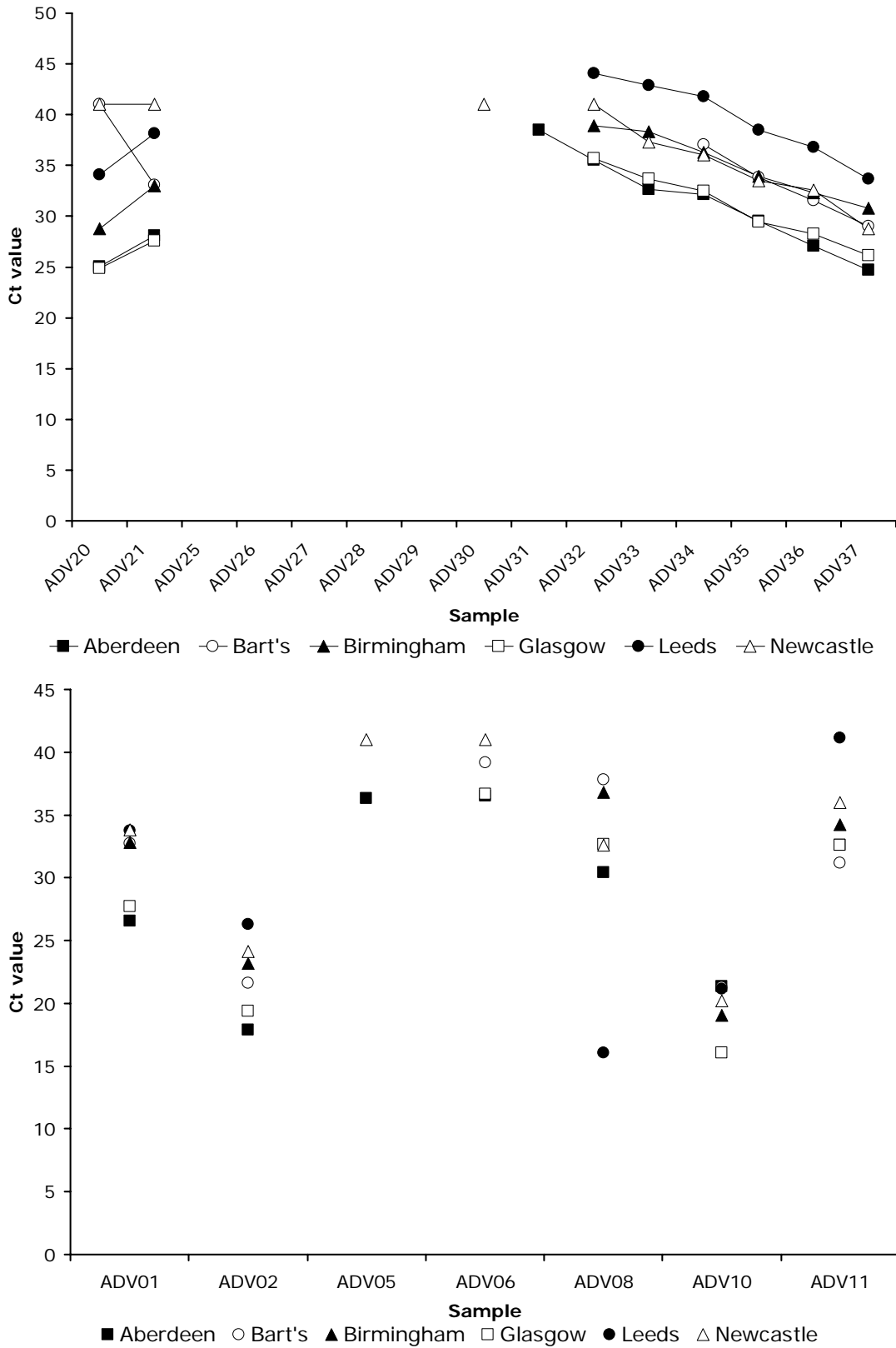


Figure 1. Graphical representation of C_t results from dilutions of nucleic acid extracted from (top) tissue culture-grown virus and (bottom) clinical samples

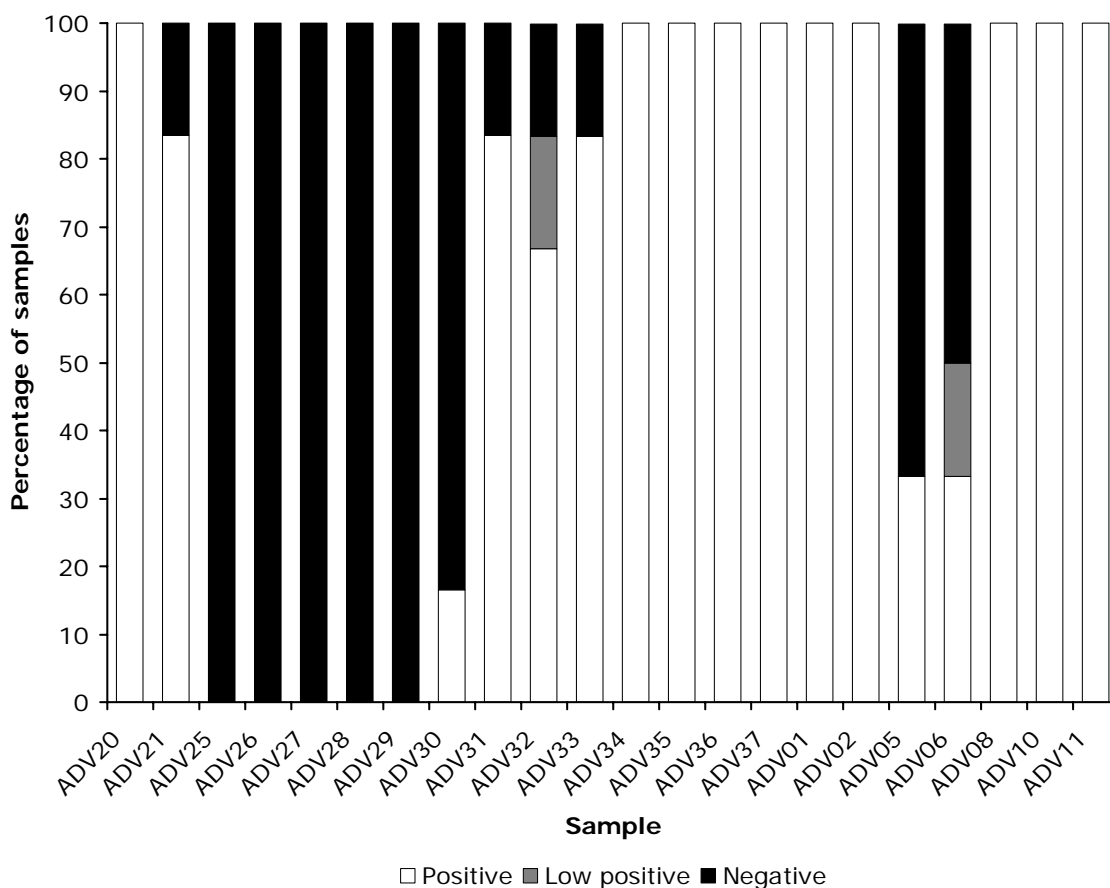


Figure 2. Distribution of final results from the panel specimens expressed as percentages

Conclusion

A range of in-house assays for the detection of adenovirus are in use across the United Kingdom (Appendix 1a). In general terms, differences between the data generated by the assays under assessment were not marked. Of all the laboratories which submitted SOPs, Aberdeen SVU performed best. Their assay was able to detect a lower dilution than any other. Additionally, Aberdeen's assay (together with Newcastle's) detected DNA in the negative clinical sample; however, additional work is required to be certain if this represents a genuine increased sensitivity.

Appendix

Appendix1a. Details of SOPs for the detection of adenovirus submitted by participating laboratories

Source	Reference	Platform	Detection method	Gene target	Assay volume
Leeds	Echavarria, M. <i>et al.</i> (1998) <i>J Clin Micro</i> 38 3323-3326	Lightcycler	DLP*	Hexon	5µl
Birmingham	Heim, A. <i>et al.</i> (2003) <i>J Med Virol</i> 70 228-239	Lightcycler	DLP	Hexon	5µl
Bart's	Heim, A. <i>et al.</i> (2003) <i>J Med Virol</i> 70 228-239 (modified)	Rotor-Gene	DLP	Hexon	24 µl
Aberdeen	Heim, A. <i>et al.</i> (2003) <i>J Med Virol</i> 70 228-239	ABI 7500	DLP	Hexon	10µl
Glasgow	Heim, A. <i>et al.</i> (2003) <i>J Med Virol</i> 70 228-239	ABI 7500	DLP	Matrix [†]	10µl
Newcastle		Lightcycler	DLP	-	5µl

*dual-labelled probe

Appendix 1b. Panel details and C_t results by laboratory for diluted tissue culture material

Panel No.	Extract method	Dilution	Laboratory*						
			WOS [†]	Abe	Bart's	Bir	Gla	Lee	New
ADV20	B [‡]	1	26.61	25.03	>41	28.76	24.84	34.08	>41
ADV21	B	1:5	28.15	28.06	33.11	33.03	27.57	38.17	neg (>41)
ADV25	B	1:500	neg	neg	neg	neg	neg	neg	neg
ADV26	B	1:1000	neg	neg	neg	neg	neg	neg	neg
ADV27	B	1:5000	neg	neg	neg	neg	neg	neg	neg
ADV28	B	1:10000	neg	neg	neg	neg	neg	neg	neg
ADV29	Q ^{††}	1:10000	neg	neg	neg	neg	neg	neg	neg
ADV30	Q	1:5000	neg	neg	neg	neg	neg	neg	>41
ADV31	Q	1:1000	neg	38.52	neg	neg	neg	neg	neg
ADV32	Q	1:500	neg	35.5	neg	38.94	35.72	44.06	>41
ADV33	Q	1:100	37.23	32.62	neg	38.3	33.63	42.88	37.32
ADV34	Q	1:50	34.28	32.19	37.07	36.3	32.48	41.77	36.04
ADV35	Q	1:10	31.16	29.49	33.87	33.92	29.41	38.48	33.49
ADV36	Q	1:5	28.74	27.1	31.53	32.35	28.28	36.79	32.59
ADV37	Q	1	27.15	24.7	28.99	30.79	26.10	33.68	28.76

*Abe – Aberdeen; Bir – Birmingham; Gla – Glasgow; Lee – Leeds; New - Newcastle

[†]original result determined in WOSSVC

[‡]BioMérieux easyMAG

^{††}Qiagen BioRobot 9604

Appendix 1c. Panel details and C_t results by laboratory for clinical samples

Panel No.	Extract method	Laboratory*						
		WOS [†]	Abe	Bar	Bir	Gla	Lee	New
ADV01	Q [‡]	29.6	26.58	32.72	32.8	27.69	33.76	33.82
ADV02	Q	19.3	17.92	21.61	23.2	19.38	26.3	24.12
ADV05	Q	neg	36.34	neg	neg	neg	neg	>41
ADV06	Q	38.5	36.54	neg (39.18)	neg (39.84)	36.64	neg	>41
ADV08	Q	34.1	30.41	37.81	36.8	32.66	38.0	37.98
ADV10	Q	17.1	21.37	21.3	19.02	16.03	21.15	20.17
ADV11	Q	32.2	32.57	31.17	34.2	32.62	41.11	35.97

*Abe – Aberdeen; Bir – Birmingham; Gla – Glasgow; Lee – Leeds; New - Newcastle

[†]original result determined in WOSSVC

[‡]Qiagen BioRobot 9604