Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2017; 5(5C):1926-1930 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2017.v05i05.043

Original Research Article

Monitoring Qualitative Non-Standard Esoteric EIA tests by the Alternative Performance Assessment (APA)

Clare Arnold-Maclean[1], Stephen Mortlock[2]
[1]Quality Control Specialist Q[2] Solutions (formerly Quest Diagnostics), Quest House, 125-135 Staines Road Hounslow, TW3 3JB
[2] Pathology Manager, Nuffield Health, Guildford Hospital, Stirling Road, Guildford, Surrey, GU2 7RF

***Corresponding author**
Dr Stephen Mortlock
Email: stephen.mortlock@nuffieldhealth.com

Abstract: More and more immunoassays have been developed using monoclonal antibodies which can quantify many different antigens simultaneously in highly complex immunoassays but, a problem in many laboratories which may test esoteric type assays is a method to quality control the assay. It is usually possible to find extra controls separate from the kit to be run with each assay, but this is only measuring the process and the ability to evaluate agreement between measurement methods or between observers is equally important. Many of the questions like limit of detection, correlation with known samples and sensitivity will have been answered during the validation of the assay but how to monitor the assay on a regular basis? The Alternative Performance Assessment Programme (APA) has been shown to be a useful tool for monitoring quantitative assays with no external schemes and in this study the esoteric qualitative assays were evaluated to see if this was also an acceptable method. Agreement was found to be between 96-100% for a range of esoteric assays.
Keywords: Alternative Performance Assessment Programme, Quality Control, EIA

INTRODUCTION

The goal of any laboratory is to provide a quality service to the clinicians by producing accurate, precise, relevant and comprehensive data that can be applied to the medical management of patients. Since its inception in 1992 Clinical Pathology Accreditation has provided formal independent recognition that a laboratory is competent in performing specific tasks [1, 2]. Quality assurance in laboratory medicine includes: a) constant checking of test reliability by internal quality control (IQC), b) external quality assessment (EQA) by an independent agency to check performance of a number of laboratories at intervals in order to obtain a retrospective indication of their performance, and c) proficiency control by supervision of pre-test and post-test phases of laboratory work, from specimen collection to delivery of report to the clinician [3-7]. In the UK the majority of laboratories are aware of the importance of EQA and to a greater or lesser extent they all perform some EQA procedures.

But, in the last decade, more and more immunoassays have been developed using monoclonal antibodies which can quantify many different antigens simultaneously in highly complex immunoassays quite often means that there is no recognised scheme to monitor the sensitivity and specificity of the assay [8-10]. But can the same system be used to monitor qualitative assays?' This study was carried out at the Q2 Solutions laboratory to determine the efficacy of the APA for these assays. This has been a problem in laboratories which may test esoteric type assays for Clinical Trials (oxidised LDL, MMP9 and some of the Interleukins), it is usually possible to find extra controls separate from the kit to be run with each assay, but this is only measuring the process. But is the assay fit for purpose? Many of the questions like limit of detection, correlation with known samples and sensitivity will have been answered during the validation of the assay but how to monitor the assay on a regular basis?

MATERIALS AND METHODS:

The Alternative Performance Assessment Programme (APA) has been shown to be a useful tool

for monitoring quantitative assays with no external schemes [11, 12].

Each qualitative assay is challenged periodically using the APA, high volume assays will be challenged more frequently than low volume ones. For each challenge, five patient samples that have been previously tested are removed from long term storage (-80°C). Whenever possible, two positive samples and three negatives are used for each challenge. The samples are quickly thawed and then split into two aliquots, giving two sets of five samples. One set is labelled A1-A5 and the second set is labelled B1-B5 and both are refrozen (-80°C). On the first day of testing set A is removed from storage, thawed, processed and assayed along with the routine samples. Set B is removed from storage and tested with the next batch of routine samples usually 24-48 hours later and if possible by a different operator. The results are then compared, evaluated and reviewed by the QA department and the laboratory. For qualitative and semi-quantitative tests the minimum passing score is 100% and 80% respectively. Alternative evaluation criteria may be used at the discretion of the Laboratory/Medical Director. Any non-conformances are fully investigated by the Quality assurance department.

The qualitative esoteric assays challenged by the APA were:-

Saliva Cotinine

The samples were processed and assayed using a standard Saliva Cotinine Assay (Cozart® Oral Fluid Microplate EIA). Although the assay was a quantitative assay, the client had requested that the results were submitted as qualitative using the following criteria, <7 ng/mL (Negative), 7-13 ng/mL (Equivocal) and >13 ng/mL (Positive) as the amount of cotinine in the sample was not part of the insurance criteria [13]. The APA reflected this requirement and the challenge were reported as a qualitative assay.

Saliva HIV:

The HIV assay used was the BioRad Genscreen™ Ultra HIV Ag-Ab as its predecessor (Genetic Systems HIV-1/HIV-2 plus O EIA) had been used by other laboratories to good effect. This method had been shown to have high sensitivity and specificity (97.0% and 99.7% respectively) when used with oral fluids and had been validated by Quest Diagnostics [14, 15].

Herpes simplex virus Direct Antigen test:

Clinical swabs of lesions were tested using a direct antigen immunoassay (Oxoid IDEIA™ Herpes Simplex Virus) kit. This is an amplified enzyme immunoassay which had been validated by Quest Diagnostics with an overall sensitivity and specificity of 96.3% and 92.1% respectively [16].

Human Papillomavirus:

The *digene* HC2 HPV DNA Test uses an RNA probe cocktail as part of a Hybrid Capture 2 technology to detect 13 high-risk and 5 low-risk HPV genotypes [17]. This assay was only challenged one time prior to an external proficiency scheme being introduced.

***Helicobacter pylori* (as part of the Gastropanel assay) [18]**

The GastroPanel® assays from biohit (Laippatie 1, 08800, Helsinki, Finland) is a set of three assays (Pepsinogen I, Gastrin 17 and *Helicobacter pylori*) and the results use an algorithm which can provide information about the stomach health and about the function of the stomach mucosa. Both the Pepsinogen I and Gastrin 17 are quantitative assays but he *H.pylori* assay however is qualitative.

***Mumps IgG*:**

The samples were processed using the Grifols Mumps assay on the Grifols Chorus.

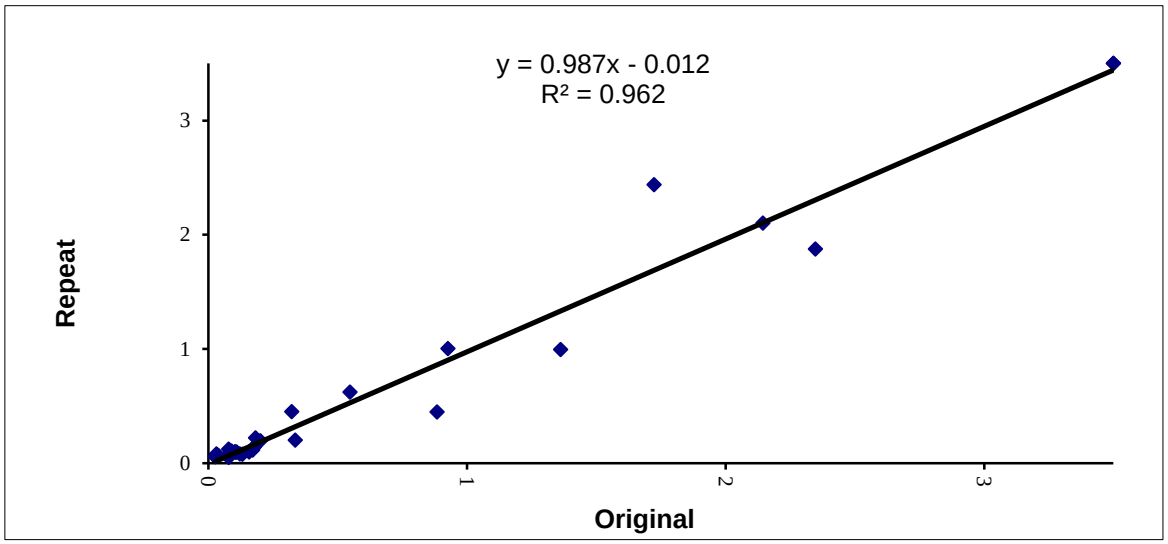
RESULTS:

With two exceptions (HSV and *H. pylori*) the APAs gave complete agreement (100%) for all of their challenges (Table 1). The failure of the *Herpes simplex* antigen was investigated and it was found that the original result, although positive was quite close to the cut-off. And when repeated went below the cut-off to give a negative results and a non-conformance (4%). A retrospective review of all of the *Herpes simplex* antigen challenges was carried out to see if this was a trend for all of the samples being used for the challenges. As this was a qualitative assay the absorbance values were analysed using a Deming regression plot and the Bland-Altman bias plot (Graph 1). To see if this was a stability issue the results were compared against the original data using ANOVA analysis (Graph 2).

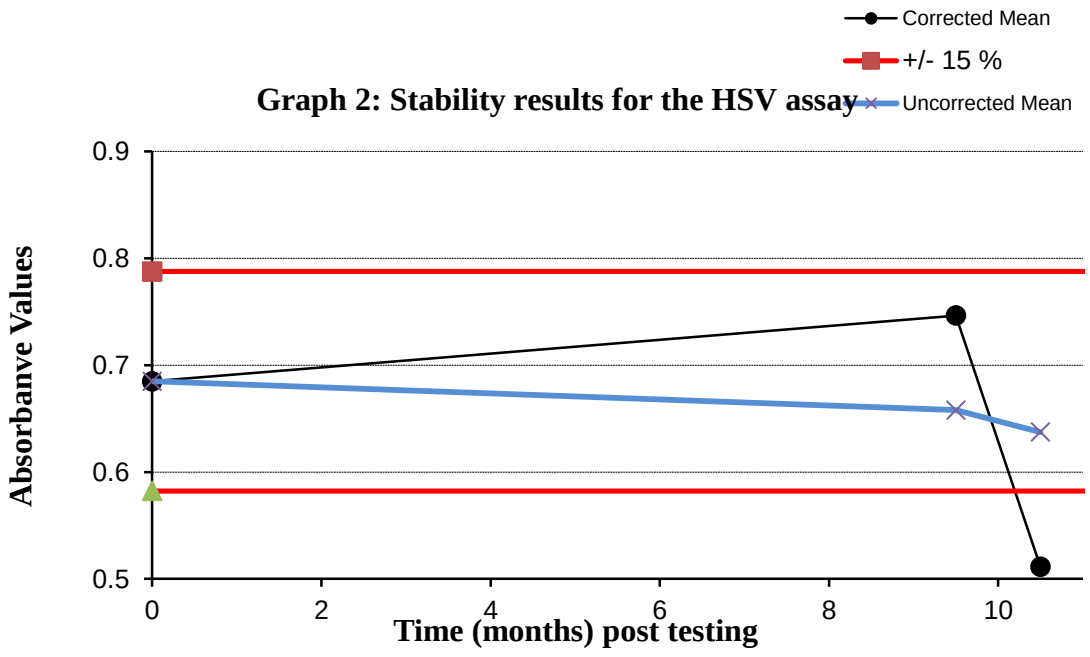
The *H.pylori* scores were a simple Positive or Negative result (anything >30 EIU was classed as Positive) and during this time there was a 96% concordance (48/50). The laboratory made one sample Positive and biohit made this negative, another sample was the reverse.

Table 1: Results of the APA Challenges.

Assay	Number of Times Challenged	Number of Results	%Agreement
Saliva Cotinine	12	60	100%
Saliva HIV	15	75	100%
HSV Antigen	6	30	96%
HPV	1	5	100%
Helicobacter pylori	10	50	96%
Mumps IgG	2	10	100%



Graph 1: Absorbance values of the *Herpes simplex* challenge samples.



Graph 2: Stability results for the HSV assay

Graph 2: Stability of HSV Antigen Assay

DISCUSSION:

Immunoassays are essentially easy to perform, cost-effective, produce highly sensitive and specific results, and allow the medical laboratory professional the ability to report accurate results in a timely manner. It is however, very important to be able to monitor the performance and in some cases there are no external proficiency schemes available.
Overall the APA challenges for all of the qualitative esoteric assays gave good agreement (100%).

In this instance the missed positive *H.pylori* result could have had repercussions for a real patient. The report would have been classed as a 'normal function of gastric mucosa' and the patient may not have received treatment. Studies have shown that screening and treatment for *H. pylori* infection significantly reduces the risk of ulcers with no requirement for maintenance therapy but the results of some serology tests were classed as indeterminate in 12.4% of patients and perhaps a "gray zone" result is a significant limitation of serological tests [19, 20]. Certainly, *H. pylori* infection induces mucosal inflammation in the stomach. Infected patients have shown a wide variety of systemic antibody responses, possibly leading to several indeterminate results in serological tests. In cases with indeterminate results, other tests should be performed to determine the status of *H. pylori* infection. In addition, the accuracy of serological tests might vary between different races and geographic regions, possibly due to different antigenic properties of local bacterial strains and antibodies of commercial kits used for the diagnosis of *H. pylori* infection. The usefulness of a serological assay should be assessed in a local setting. But successful eradication of *H. pylori* results in significantly lower endoscopic recurrence rates for gastric ulcer patients either with or without administration of non-steroidal anti-inflammatory drugs (NSAID) [21, 22].

The investigation of the *Herpes simplex* antigen assay absorbance values showed that on retesting there had been a decrease in absorbance after the freeze thaw cycle. It has long been known that repeated freezing and thawing of serum or plasma can have detrimental effects on certain analytes [23, 24]. A study using a PCR technique for *herpes simplex* using lesion fluid did find that after freeze-thawing the sample the inhibition levels increased but this inhibition disappeared when the sample was diluted [25]. In this instance, the agreement between the A sample absorbance values and the B sample values was 96% (29/30) with an R^2 of 0.962 and an average bias of 3.11%. No difference in the absorbance values was observed for the samples by ANOVA, with P= 0.997. Regression analysis did not show any differences as well, with a slope of 0.977 (SE=0.042), y-intercept of -0.011 (SE= 0.051), for the fresh and A sample comparison; a slope of 0.969 (SE=0.053) and y-intercept of -0.026 (SE=0.063) for the fresh and B sample comparison; and with slope of 0.986 (SE=0.037) and y-intercept of -0.011 (SE=0.043) for the A and B comparison. The three regression graphs had a correlation coefficient of at least 0.95. So, although the corrected mean was outside the range the ANOVA analysis suggested that the freeze thaw had not been detrimental to the sample stability and the results were acceptable.

It seems that the freeze thawing was not instrumental in the failure of this sample. Looking at the failed sample on its own showed that the initial positive was only just above the cut-off value as designated by the kit (average of the negative absorbance values plus 0.150) with a value of 0.256/0.239 for the A sample compared to 0.201/0.225 for the B sample. The initial sample had an absorbance reading of 0.277/0.241. So it is critical that when choosing a sample for the APA challenge the absorbance value is sufficiently high to prevent a similar failure.

CONCLUSION:

In conclusion, however the APA programme worked very well for all of the qualitative esoteric assays and was a suitable alternative to an external proficiency test until such time these assays become part of a recognized scheme.

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