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Influence of expiration date on analytical performance of rapid tests for HIV diagnosis

Influência do prazo de validade no desempenho analítico de teste rápido para o diagnóstico do HIV

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ABSTRACT

Introduction: Rapid tests (RTs) are ideal to provide laboratorial results within 30 minutes in a variety of situations and locations, even in underserved communities. Like any other laboratory input, they can only be used within the expiration date. **Objective:** The aim of this study was to compare the analytical performance of the RT Interkit HIV 1 and 2® (Interteck Katal - Belo Horizonte, Brazil) at two different times, two years before and one week before the expiration date. **Material and methods:** Two hundred serum samples were used, from individuals over 18 months of age, of both sexes, previously tested by electrochemiluminescence and Western Blot for HIV infection, distributed in two groups: reactive (n = 100) and non-reactive (n = 100). **Results:** The RT showed similar and satisfactory diagnostic accuracy in the two analyzed moments, with no statistical difference. **Conclusion:** The RT Interkit HIV 1 and 2® showed stable analytical performance both at the beginning and the end of its validity term.

Key words: HIV; diagnosis; date of validity of products; sensitivity; specificity.

RESUMO

Introdução: *Os testes rápidos (TR) são ideais para fornecer resultados laboratoriais em até 30 minutos, em diversas situações e locais, mesmo em comunidades mais carentes. Como qualquer outro insumo laboratorial, eles só podem ser utilizados dentro do prazo de validade.* ***Objetivo:*** *O objetivo deste estudo foi comparar o desempenho analítico do TR Interkit HIV 1 e 2® (Interteck Katal, Belo Horizonte, Brasil) em dois momentos: dois anos antes e uma semana antes do vencimento.* ***Material e métodos:*** *Duzentas amostras de soro de indivíduos com mais de 18 meses de idade, de ambos os sexos, foram utilizadas. Elas foram previamente testadas por eletroquimioluminescência e Western Blot para a infecção por HIV e distribuídas em dois grupos: reagentes (*n = *100) e não reagentes (*n = *100).* ***Resultados:*** *O TR apresentou acurácia diagnóstica satisfatória e semelhante nos dois momentos analisados, sem diferença estatística.* ***Conclusão:*** *O TR Interkit HIV 1 e 2® apresentou desempenho analítico estável, tanto no início quanto no final do prazo de validade.*

Unitermos: *HIV; diagnóstico; prazo de validade de produtos; sensibilidade; especificidade.*

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RESUMEN

Introducción: *Las pruebas rápidas son ideales pues dan resultados en hasta 30 minutos, en diversas situaciones y locales, incluso en localidades carentes. Como cualquier otro insumo de laboratorio, solo pueden ser utilizados dentro de la fecha de caducidad.* ***Objetivo:*** *Comparar el desempeño analítico de la PR Interkit HIV 1 y 2® (Interteck Katal, Belo Horizonte, Brasil) en dos momentos: dos años antes y una semana antes del vencimiento de la fecha de caducidad.* ***Material y método:*** *Se utilizaron doscientas muestras de sueros de individuos mayores de 18 meses de edad, de ambos sexos. Las muestras fueron previamente probadas por electroquimioluminiscencia y Western Blot para infección por VIH y distribuidas en dos grupos: reactivas (*n = *100) y no-reactivas (*n = *100).* ***Resultados:*** *La PR presentó precisión diagnóstica satisfactoria y semejante en ambos momentos analizados, sin diferencia estadística.* ***Conclusión:*** *La PR Interkit HIV 1 y 2® presentó desempeño analítico estable, tanto al principio cuanto al final de su fecha de caducidad.*

Palabras clave: *VIH; diagnóstico; fecha de caducidad de productos; sensibilidad; especificidad.*

INTRODUCTION

Rapid tests (RTs)[1], also termed point-of-care tests (POCTs), are excellent because they provide results in up to 30 minutes, in several situations and locations (they can be used in non-laboratory environments by capable personnel[2]) and because they allow to broaden access to diagnosis in underserved communities[3, 4]. In Brazil, RTs have been part of human immunodeficiency virus (HIV) screening since 2001[5].

According to regulation no. 8.137 from 1990, it is expressly prohibited to commercialize and use products out of date[6]. As stated by Agência Nacional de Vigilância Sanitária (Anvisa), the use of inputs for diagnostic purposes in Brazil is ruled by the Resolution of the Collegiate Directorate (RDC) 302[7], RDC 11[8] and RDC 36[9], which have several forms for the functioning of clinical laboratories, among them, the adequate use of reagents and inputs, which must respect the usage recommendations by the manufacturer, preservation conditions, storage and expiration dates; the use of reagents and inputs and their revalidation after expiration date for diagnostic purposes[7]. Good laboratory practices are also aims of accreditation programs of laboratory quality, such as the Programa de Acreditação de Laboratórios Clínicos (PALC) of Sociedade Brasileira de Patologia Clínica/ Medicina Laboratorial (SBPC/ML)[10].

Part of the RTs produced to diagnose HIV infection, for example, present expiration dates that can range from one to two years, as determined by manufacturers. The stability of those kits close to the expiry date is a common concern, because the risks posed by the use of expired laboratory inputs are several, principally the inadequate functioning of the reagent, what can generate false positive or false negative results, besides cross-reactivity or unspecified reactions[8]. Facente *et al*., in 2009, had already observed significant increase of false positive results in oral RT for HIV used in the last month before expiration[11].

Regrettably, literature lacks works assessing the analytical performance of laboratory tests regarding the validity date in daily clinical laboratory practice; this responsibility is placed on manufacturers.

The objective of this work was to compare the analytical performance of a RT for diagnosis of HIV infection in two distinct moments: in the beginning and one week before expiration date, in blood samples with previously defined serological pattern.

MATERIAL AND METHODS

Analytical, observational, and concordance study, in which the analytical performance of the Interkit HIV 1 and 2® kit (Interteck Katal, Belo Horizonte, Brazil)[12] was analyzed in two moments: A) two years before expiration date; B) one week before expiration date. Lot number: 3006/16000001; expiration date: May 23, 2018.

Two hundred serum samples of individuals older than 18 months of age, from both sexes – distributed in two groups: reactive ($n = 100$) and non-reactive ($n = 100$) – were previously tested with electrochemiluminescence immunoassay [(ECLIA) – screening] and Western blot [(WB) – confirmatory] for HIV infection.

Samples with results above the cutoff value of the ECLIA method (HIV Combi – HIV-1 antigen and anti-HIV-1 and anti-HIV-2 total antibodies, Roche Diagnostics, Mannheim, Germany)[13] were considered reactive (index = 1); at WB (New Lav Blot I, BioRad, Marnes la Coquette, France)[14], the presence was necessary of at least one of the combinations of the bands: p24; gp41;

gp120/gp160, according to flowchart number 6 of ordinance 29, from December17, 2013[15].

Samples with results below the cutoff value at ECLIA and absence of bands at WB were considered non-reactive.

They were considered:

• true positive (TP) – samples reactive at ECLIA, WB and RT;

• true negative (TN) – samples non-reactive at ECLIA, WB and RT;

• false positive (FP) – samples non-reactive at ECLIA and WB, but reactive at RTs;

• false negative (FN) – samples reactive at ECLIA and WB and non-reactive at RTs.

For each testing moment, the following diagnostic accuracy measures were calculated: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR), negative likelihood ratio (-LR), and accuracy.

The tests took place at the laboratory of the Medical School of Universidade Federal de Minas Gerais (UFMG), according to instructions by the manufacturer.

The statistical analysis was conducted in the IBM program SPSS *Statistics for Windows*, version 19.0. Analytical performance and result agreement were calculated using paired *t* test, with a 95% confidence interval (CI) and significance level of $p < 0.05$.

The study was approved by the Research Ethics Committee of UFMG (report CAAE 47246115.6.0000.5149) and rendered the free informed consent unnecessary.

RESULTS

The analytical performance of the Interkit HIV 1 and 2® kit in both testing moments (A and B) is presented in the **Table**.

The RT presented diagnostic accuracy satisfactory and similar in both analyzed moments, with no statistical difference. In other words, the tests conducted close to their expiration date presented stable analytical performance when compared with the results of those performed soon after manufacturing date.

TABLE – Comparison of analytical performance of the Interkit HIV 1 and 2 kit in moments A and B

Analytical performance	Moment A (95% CI)	Moment B (95% CI)	$p < 0.05^{*}$
Sensitivity (%)	100 (-)	100 (-)	1
Specificity (%)	98 (93-99.8)	98 (93-99.8)	1
PPV (%)	96.7 (94.7-98.7)	98 (93-99.8)	0.757
NPV (%)	100 (-)	100 (-)	1
Positive likelihood ratio (%)	0.5 (0.43-0.57)	1 (-)	0.5
Negative likelihood ratio (%)	0 (-)	0 (-)	1
Accuracy (%)	99 (97.6-100)	100 (-)	0.887

*CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value: negative predictive value; *paired* t *test.*

DISCUSSION

As the RT can be used outside a laboratory setting, but by properly trained staff, the information present in the usage instructions must be clear and objective, especially the product expiration date. Expiration date of a product is the period of time after manufacturing in which a product keeps quality and stability of its characteristics, and it can be used for a certain function, within standards established by law and/or acceptable by the consumer[16-18]. Expiration date corresponds to the time necessary to reach the threshold levels of degradation products or loss of a product initial active content[16-18].

In order to assess the stability of a product, several tests are conducted using validated quantitative analytical methods that can detect time changes in chemical, physical or microbiological properties of the product, which are specific so that the content of the active ingredient, the degradation products and other components of interest can be measured precisely, with no interference[19].

The stability of a product can be influenced by different factors, such as its nature (physical-chemical-biological constituents), the environment (temperature, pressure, humidity, and light), storage (whether inside or outside the original packaging, whether refrigerated, frozen, or at room temperature), transportation (physical damages), handling (whether adequate or not, according to recommendations by the manufacturer), among others. Therefore, the loss of product stability is, in general, a dynamic process, whose risks do not appear immediately or soon after the expiring date informed by the manufacturer[18, 20, 21].

According to Klarkowski *et al*. (2013)[22], the test specificity can vary according to location (within and between countries) and over time; those variations are not characteristic of a single test. The possible explanations for such variability are, mainly, changes in performance (sensitivity or specificity) or in the quality of a certain test. Alterations in sensitivity, due to variations in the test capacity to detect early seroconversion, or to viral genetic diversity of HIV, are other potential causes for disagreement.

There are also studies demonstrating that, as the RT kits age, general testing specificity decreases[11]. Such a fact was not observed in this study, because the evaluated RT presented stable analytical performance when tested at the beginning and end of its validity date. A possible explanation for this disagreement could be related with other factors that also influenced the kit stability, besides the expiration date itself, such as storage conditions, transport, temperature, humidity, light, training and even quality of the team

handling the kits[18]. The stability of RT performance also varies according to the quality of the inputs used in their production and the quality and representativeness of the tested biological samples[19]. Therefore, it is health professionals' responsibility to manage their diagnostic inputs and control the interfering factors to ensure good practices in laboratory routine[7-10].

CONCLUSION

The RT Interkit HIV 1 and 2® showed stable and satisfactory analytical performance in the two distinct testing moments: at the beginning and end of its expiration date.

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