

Validación y verificación de un protocolo *in house* con nanopartículas magnéticas para la extracción de ácidos nucleicos de SARS-CoV-2

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Código estudiantil: 2021120540508

Trabajo de grado presentado como requisito para optar el título de:
Magister en Genética

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RESUMEN

En la pandemia generada por COVID-19 causó crisis sanitaria a nivel mundial, principalmente por el desabastecimiento de insumos médicos entre ellos las pruebas diagnósticas de SARS-CoV-2, el problema se agudizó en países como Colombia, y otros en América Latina. Los países en desarrollo, la financiación en los sistemas de atención médica es limitada, lo anterior generó consecuencias que provocaron el aumento en la mortalidad y limitó la detección temprana del virus.

En la búsqueda de herramientas importantes que permitieran abordar la problemática, favorecer la investigación biomédica básica y el diagnóstico clínico, se diseñó y optimizó un método de extracción de ácidos nucleicos *in house* el cual permita mejorar la accesibilidad y contribuir a la independencia biotecnológica en el contexto local. En el presente estudio tuvo como objetivo presentar un método de síntesis por coprecipitación de nanopartículas magnéticas de hierro sin recubrimiento, basado en la síntesis de BOMB (Oberacker et al., 2019), diseñadas específicamente para la recuperación de material genético, garantizando tanto su eficiencia como su calidad. Para asegurar la viabilidad de este enfoque, evaluó la robustez método de síntesis y se estandarizó el proceso de creación de nanopartículas magnéticas, así como se evaluó la efectividad de las soluciones de

lisis que hacen parte del kit de extracción de ácidos nucleicos. Además, se realizó una estimación de costos del kit in house con el fin de evaluar su viabilidad, obteniendo el kit in house resultó un 93.7 % más económico, en comparación con el precio del kit comercial, además el kit in house mostró mejor rendimiento con relación a permitir realizar mayor número de pruebas.

Para la validación del método se realizó por medio de RT-qPCR a partir de muestras 21 negativas y 21 muestras positivas de SARS-CoV-2. Es importante destacar que el kit in house presenta mejor concentración en la recuperación de AN, en comparación al kit comercial Zymo. En los ensayos de selectividad se obtuvo que el protocolo in house presentó una especificidad del 100% y una sensibilidad del 95% para la detección de SARS-CoV-2. Los parámetros de linealidad indicaron que el método proporciona resultados consistentes y predecibles a través de un rango de diluciones. El punto de corte Ct para el RT-qPCR de SARS-CoV-2 del kit ENA *in house* es de 37,8 con 90% de confianza. En los ensayos de precisión intermedia del kit los resultados mostraron poca variabilidad entre los analistas, indicando con ello resultados consistentes, precisos y reproducibles en muestras biológicas. Posteriormente, el método de extracción de ácidos nucleicos se valida consolidando así la utilidad y aplicabilidad del kit desarrollado para uso institucional.

La organización mundial de la salud (OMS), la Organización Panamericana de la Salud (OPS), países como: Argentina, Brasil, Ecuador, instituciones nacionales como el ministerio de Ciencia y Tecnología, promueven como objetivo común la búsqueda de la autonomía biotecnológica, que incentiven a la producción nacional de tecnologías en salud para el país y para América Latina. Para de esta forma ampliar las capacidades de producción local, como herramientas garantes de acceso que permitan atender las necesidades en salud y las emergencias en salud pública.

Palabras clave: Nanopartículas magnéticas, extracción de ácidos nucleicos, validación.

ABSTRACT

The pandemic generated by COVID-19 caused a worldwide health crisis, mainly due to the shortage of medical supplies, including diagnostic tests for SARS-CoV-2, and the problem worsened in countries such as Colombia and others in Latin America. In developing countries, funding in health care systems is limited, which has had consequences that have led to increased mortality and limited early detection of the virus.

In the search for important tools to address the problem, promote basic biomedical research and clinical diagnosis, an *in-house* nucleic acid extraction method was designed and optimized to improve accessibility and contribute to biotechnological independence in the local context. The present study aimed to present a method for the co-precipitation synthesis of uncoated magnetic iron nanoparticles based on BOMB synthesis (Oberacker et al., 2019), specifically designed for the recovery of genetic material, ensuring both efficiency and quality. To ensure the feasibility of this approach, the robustness of the synthesis method was evaluated and the process of creating magnetic nanoparticles was standardized, as well as the effectiveness of the lysis solutions that are part of the nucleic acid extraction kit was evaluated. In addition, a cost estimation of the *in-house* kit was made in order to evaluate its viability, obtaining that the *in-house* kit was 93.7 % cheaper, compared to the price of the commercial kit, in addition the *in house* kit showed better performance in relation to allowing a greater number of tests to be performed.

For the validation of the method, RT-qPCR was performed on 21 negative and 21 positive samples of SARS-CoV-2. It is important to note that the *in-house* kit presented better concentration in AN recovery compared to the commercial Zymo kit. In the selectivity tests, the *in-house* protocol showed a specificity of 100% and a sensitivity of 95% for the detection of SARS-CoV-2. Linearity parameters indicated that the method provided consistent and predictable results across a range of dilutions. The cutoff point Ct for SARS-CoV-2 RT-qPCR from the in-house ENA kit is 37.8 with 90% confidence. In the intermediate precision assays of the kit the results showed little inter-analyte variability, thereby indicating consistent, accurate and reproducible results in biological samples.

Subsequently, the nucleic acid extraction method was validated, thus consolidating the usefulness and applicability of the kit developed for institutional use.

The World Health Organization (WHO), the Pan American Health Organization (PAHO), countries such as Argentina, Brazil, Ecuador, national institutions such as the Ministry of Science and Technology, promote as a common objective the search for biotechnological autonomy, which encourages the national production of health technologies for the country and for Latin America. In this way, to expand local production capacities, as tools that guarantee access to meet health needs and public health emergencies.

Key Words: Magnetic nanoparticles, nucleic acid extraction, validation.

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