## DIRECT IMMUNOFLUORESCENCE ASSAY FOR H1N1 DIAGNOSIS

It is with great interest that I read the recent article by Pianciola et al. titled "Direct Immunofluorescence Assay Performance in Diagnosis of the Influenza A (H1N1) Virus," published in Volume 27, Number 6, of this journal. The authors concluded that this test had "a sensitivity of 44.4%, a specificity of 99.6%, a positive predictive value of 95.2%, and a negative predictive value of 90.7%" (1). However, there are problems with this study that should be mentioned.

First, the study had very few subjects ($n$ = 293), which limits the implications of the results. Indeed, the sensitivity and specificity of the test are directly related to the number of subjects.

Second, the difference between the positive results obtained with DFA and those obtained with PCR differs greatly from that of a previous study (2). This might be due to an actual difference or it might reflect a quality control issue. Piaciola et al. did not address the quality control process of the test in their report.

It should be noted that a recent study by Pollock et al., conducted at a standard reference laboratory with good quality control, showed that "DFA tests can effectively rule out infection due to novel H1N1 virus" (3).

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## REFERENCES

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## *RESPUESTA DE AUTOR*

Hemos leído con interés los comentarios de Viroj Wiwanitkit sobre nuestro artículo "Desempeño de la prueba de inmunofluorescencia directa en el diagnóstico del virus Influenza A (H1N1)", publicado en el volumen 27, número 6 de esta revista. El objetivo del trabajo fue aportar datos de nuestros laboratorios acerca de la sensibilidad de un método diagnóstico sobre el que se habían hallado resultados contradictorios.

Concordamos en que es deseable estudiar una muestra numerosa y creemos que 293 casos constituyen un tamaño considerable para extraer algunas conclusiones "en las condiciones analizadas" —según consta en nuestro estudio. Debe tenerse en cuenta que el trabajo de Pollock y colaboradores, citado por Wiwanitkit, que revela hallazgos contrarios a los nuestros, solo evalúa 112 casos (1). Por otra parte, si bien es cierto que la fortaleza de los datos de sensibilidad y especificidad de una metodología analítica será mayor cuantos más casos se hayan evaluado, el valor en sí de estos parámetros, opuesto a lo que se afirma en la carta, no tiene relación directa con el tamaño de la muestra.

Respecto a los controles de calidad de la metodología, es importante destacar que las muestras fueron estudiadas en el Laboratorio de Referencia Provincial en Virus Respiratorios, que cuenta con más de 15 años de experiencia en la técnica de inmunofluorescencia directa (DFA), avalada por el procesamiento de más de 30 000 especímenes en ese período. Asimismo, se siguieron estrictamente las instrucciones metodológicas del fabricante del equipo, con especial adhesión a sus exigencias acerca de los controles de calidad. El Laboratorio cuenta además con controles de calidad internos y está sometido a los controles de calidad externos de la Red Nacional de Virosis Respiratorias de Argentina.

No coincidimos con Wiwanitkit respecto a que nuestros datos difieren largamente de los trabajos previos. Como ya mencionamos, este es un tema con resultados contradictorios. De hecho, en la carta se menciona un trabajo que reveló una alta sensibilidad de DFA (93%) con solo 112 muestras, mientras que el Laboratorio Nacional de Referencia de Influenza de Argentina publicó resultados de sensibilidad de 59,5% para virus influenza A estacionales utilizando la misma prueba (1, 2). Es importante mencionar que el fabricante del equipo utilizado también hace referencia a una limitada sensibilidad del método para la detección de virus influenza A estacionales, mostrando resultados de dos estudios con DFA que dieron sensibilidades de 48,4% y 83,3% (3). Debe destacarse el trabajo de Ginocchio y colaboradores, mencionado en nuestro estudio, quienes basados en 2 861 muestras encontraron sensibilidades de DFA para detectar H1N1 de alrededor de 47% (4). La solidez de estos hallazgos —muy similares a los nuestros— basados en tan alto número de muestras nos eximen de mayores comentarios.

Finalmente, deseamos agregar que los resultados obtenidos posteriormente a la elaboración de nuestro estudio siguen avalando una limitada sensibilidad de la técnica para el diagnóstico de este virus. Por lo tanto, continuamos recomendando una interpretación cautelosa de los resultados obtenidos con DFA.

## *AUTHORS' REPLY* [1]

It is with interest that we read Viroj Wiwanitkit's comments on our article, "Direct Immunofluorescence Assay Performance in Diagnosis of the Influenza A (H1N1) Virus," published in Volume 27, Number 6, of this journal. The purpose of our study was to share data from our laboratories regarding the sensitivity of a diagnostic method that has produced conflicting results.

We agree that studying a large number of samples is best, and consider 293 samples to constitute a number adequate for drawing some conclusions "within the study's limitations," as stated in our study. It should be noted that the work of Pollock et al., cited by Wiwanitkit, which produced findings contrary to ours, analyzed only 112 cases (1). Moreover, though true that the strength of a diagnostic method's sensitivity and specificity data increases with the number of samples analyzed, contrary to what is stated in the letter, its intrinsic value is not directly related to sample size.

Regarding quality control methods, it is worth emphasizing that the samples were analyzed by the Provincial Reference Laboratory on Respiratory Viruses, which has over 15 years of experience with direct immunofluorescence assay (DFA) techniques, backed by more than 30 000 specimens processed during that time. Also, the manufacturer's instructions were followed meticulously, with special attention given to its quality control requirements. In addition, the Laboratory itself has internal quality control measures and is subject to quality control oversight by the Argentine National Network on Respiratory Viruses.

We do not concur with Wiwanitkit that our data differ greatly from that of prior works. As previously mentioned, this is an issue of conflicting results. In fact, the letter refers to a work that showed DFA to have high sensitivity (93%) with only 112 samples, while the Argentine National Reference Laboratory published results of 59.5% sensitivity for seasonal influenza type A virus using the same test (1, 2). It is important to note that the manufacturer itself acknowledges the method's limited sensitivity to seasonal influenza A virus, sharing results of two DFA studies that found sensitivity to be 48.4% and 83.3% (3). The work of Ginocchio et al., referenced in our study, must be highlighted as it was based on 2 861 samples and found DFA sensitivity of approximately 47% for H1N1 detection (4). The strength of these findings, very similar to our own and based on such a large number of samples, exempts us from further comment.

Lastly, we would like to add that results obtained since the completion of our study continue to support limited sensitivity of this method for diagnosing this virus. Therefore, we continue to recommend cautious interpretation of DFA results.

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[1] Translated by the Pan American Journal of Public Health.

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