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# Purification of *O. volvulus* Genomic DNA from *S. ochraceum* s.l. Black Fly Head or Body...

Purification of *O. volvulus* Genomic DNA from *S. ochraceum* s.l. Black Fly Head or Body Pools using the Maxwell® 16 Instrument


**ABSTRACT**

*Onchocerciasis is still a major human disease transmitted through the bites of* Simulium *black flies. A gold standard DNA isolation method from pools of black flies connected to O-150 PCR-ELISA is used to monitor the success of transmission control programs based on ivermectin treatment. Here, the new Maxwell® 16 automated method from Promega was used to purify DNA from pools of black flies. The Maxwell® 16 method was faster and less tedious compared to the standard method. No statistical difference in the entomological estimators of transmission was attained when using either of the two methods.*



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## Introduction

Onchocerciasis, a human disease caused by infection with the filarial parasite *Onchocerca volvulus*, is a major health threat worldwide. Thirty-seven to forty million people around the world have been infected with the parasite, of which 779,000 people are now blind. Concurrent with the use of Mectizan® (ivermectin) for the treatment of onchocerciasis, a sensitive O-150 PCR-ELISA (1) (2) was developed that provided the efficient, sensitive and specific tool necessary to monitor the decline and interruption of transmission achieved by successful control programs based on ivermectin treatment (2) . The current gold standard method for preparing genomic DNA from black fly pools for O-150 PCR-ELISA is phenol chloroform (P-C) extraction, followed by two rounds of adsorption to a silica matrix. This process is time-consuming and requires skilled technicians to be carried out effectively. More efficient DNA purification methods would enhance the performance of molecular detection techniques such as the O-150 PCR-ELISA. In this study, the relative effectiveness of an automated DNA purification method based on paramagnetic particles (Maxwell® 16 Tissue DNA Purification Kit; Cat.# AS1030) has been compared to and validated against the standard P-C method. The outcome of each method was used to estimate the vector transmission parameters in the local fly population at the peak *O. volvulus* transmission season lasting from February to April 1994 in Las Golondrinas, a Mexican community endemic for onchocerciasis located in the Southern Chiapas focus of Mexico.

## Mixing Experiment

Certification of elimination of onchocerciasis in the endemic foci require the collection of very large numbers of black flies (at least 10,000 per community with several communities per survey focus). Therefore, we tested the sensitivity of O-150 PCR-ELISA for the ability to detect a single *O. volvulus*-infected *Simulium ochraceum* s.l. head or body from pools that contain

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increasing quantities of uninfected heads or bodies using the genomic DNA extracted with the automated Maxwell® 16 method.

**(i) Paramagnetic Particle Method**

Different combinations of infected and uninfected fly heads or bodies were mixed as follows: 1:49, 1:59, 1:69, 1:79, 1:89, 1:99, 1:109, 1:119, 1:129, 1:139, 1:149, 1:159, 1:169, 1:179, 1:189 and 1:199. The mixed samples were inserted into the cartridge supplied with the Maxwell® 16 Tissue DNA Purification Kit (Cat.# AS1030), containing lysis buffer, wash buffer, elution buffer, elution tubes, silica-coated MagneSil® Paramagnetic Particles (PMPs) and a plunger. Individual DNA samples were purified within 45 minutes without any cross-contamination and eluted into a tube using 300µl of buffer.

**(ii) Phenol-Chloroform Method**

Eleven combinations of infected and uninfected fly heads or bodies were mixed as follows: 1:49, 1:59, 1:69, 1:79, 1:89, 1:99, 1:109, 1:119, 1:129, 1:139, and 1:149. The fly heads or bodies were placed in 1.5ml microcentrifuge tubes and rinsed three times with 95% ethanol followed by evaporation at room temperature. The samples were homogenized in 300µl of lysis buffer (100mM NaCl, 10mM Tris-HCl [pH 8.0], 1mM ethylenediamine tetra-acetic acid [EDTA], 0.1% sodium dodecyl sulphate [SDS]) along with 2µl of carrier salmon sperm DNA (250ng/µl), and incubated at 55°C for 1 hour in 100µg/ml of proteinase K solution. The lysed samples were boiled for 30 minutes in the presence of 10mM dithiothreitol (DTT) to disrupt the parasite cuticle, followed by a series of freeze-thaw steps to release the DNA from the parasites. The DNA was then purified in two cycles of extraction with 1:1 (v/v) phenol-chloroform and one with chloroform according to Rodríguez-Pérez *et al*. (3) . The genomic DNA was stored at –20°C until tested by O-150 PCR-ELISA.

**O-150 PCR-ELISA**

Three microliters of the purified genomic DNA was used as a template for PCR in 50µl total volume containing 0.5µmol/L of O-150 primer (5´-GATTYTTCCGRCGAANARCGC-3´) and 0.5µmol/L of biotinylated O-150 primer (5´-B-GCNRTRTAAATNTGNAAATTC- 3´, where B = biotin; N = A, G, C, or T; Y = C or T; and R = A or G) according to Rodríguez-Pérez *et al*. (4) .

**Validation Study under Field Conditions**

The flies were divided into two groups, one for the Maxwell® 16 paramagnetic particle method with pool size of 100 flies and the other pool with 50 flies for the P-C method. The total number of flies used in both methods was 13,400. The flies were equally divided into two groups for DNA purification based on collection site (center of the village and in the nearby coffee plantation to ensure that the wild-caught sample black fly population includes the two sites where people are exposed to black fly bites) and three different collection times: 07:00 to 09:00, 10:00 to 14:00, and 15:00 to 17:00.

**Data Analysis**

Three pair-wise Wilcoxon-type tests (a test per either head pools or body pools, and a combination of both) were performed to compare the performance of O-150 PCR-ELISA using the DNA extracted by each of the two methods. The prevalence of *O. volvulus* infection in the body pools and head pools and associated 95% confidence intervals (CIs) were determined using program Pool screen version 2.0 (5) . The proportion of infective flies (in head pools) was used to calculate seasonal transmission potential, which is the product of the seasonal biting rate, the proportion of flies carrying third stage larvae in the

transmission season (February–April 1994), and the average number of third-stage larvae in each infective fly, which was assumed to be 1.0 (2) . The seasonal biting rate was calculated as the product of the Williams geometric mean of the number of flies collected per person/day and the total number of days in the transmission season.

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**Results**

Two different DNA purification methods using experimentally prepared combinations of a single infected fly head or body and varying numbers of uninfected fly heads or bodies were compared and validated by O-150 PCR-ELISA. In the mixing experiment using the P-C method, the O-150 PCR-ELISA was able to detect the pool containing 1 infected female body and head in pools of 149, and 99 uninfected bodies and heads, respectively. In contrast, using the paramagnetic particle method, the O-150 PCR-ELISA was able to detect the pool containing 1 infected female body and head in pools of 139, and 149 uninfected bodies and heads, respectively. No statistical differences ($P>0.05$; $Z = -0.57$) were noted when comparing the performance of the O-150 PCR-ELISA using the DNA extracted from either head pools or body pools and a combination of both by the two methods. Based on these results, a pool size of 50, and 100 for the P-C and paramagnetic particle method, respectively, were selected to ensure that our methods would detect any infected fly body or head when screening the wild-caught population. The number of flies processed, and the number of positive heads and bodies are summarized in Table 1. Using the P-C method, the infection rate at 3.78 infected flies/2,000 flies (95% CI = 1.89–6.07) and 1.05 infective flies/2,000 flies (95% CI = 0.14–2.16) was estimated. Using the paramagnetic particle method, the infection rate at 2.35 infected flies/2,000 flies (95% CI = 0.83–4.06) and 1.69 infective flies/2,000 flies (95% CI = 0.45–3.13) was determined. The estimated seasonal transmission potential in Las Golondrinas in the 1994 transmission season (5.5 and 8.8 L3s per person per season using the P-C and paramagnetic particle method, respectively) was statistically similar ($P>0.05$; $Z = 0.57$) using both methods of extraction for parasite DNA in pools of flies (Table 2).

Comparison of the Number of Positive Pools for *O. volvulus* When Using Two Methods of DNA Purification from Heads or Bodies of *S. ochraceum* s.l. Flies.

| | **Phenol-Chloroform Method** | **Paramagnetic Particle Method** |
|---|---|---|
| Pool size | 50 | 100 |
| Number of flies examined | 6,700 | 6,700 |
| Number of body and head pools tested | 134 | 67 |
| Number of positive body pools | 12 | 7 |
| Number of positive head pools | 3 | 5 |

**Table 1. Comparison of the Number of Positive Pools for *O. volvulus* When Using Two Methods of DNA Purification from Heads or Bodies of *S. ochraceum* s.l. Flies.**

Prevalence of Infected and Infective Rate, and Seasonal Transmission Potential Estimated in Las Golondrinas, Mexico, When Using Two Methods of Parasite DNA Purification.

| Community/Year/ Seasonal Biting Rate | DNA Purification Method | Prevalence of Infected Rate* | Prevalence of Infective Rate* | Seasonal Transmission Potential |
|---|---|---|---|---|
| Las Golondrinas/1994/ 10,522 bites per person per season | Phenol-chloroform | **3.78** (1.89–6.07) | **1.05** (0.14–2.16) | **5.5** (0.73–11.36) |
| | Paramagnetic particle method | **2.30** (0.83–4.06) | **1.69** (0.45–3.13) | **8.8** (2.3–16.46) |

*Expressed as rate per 2,000 flies examined.
**Note:** The bold numbers represent the point estimate for the value in question and the numbers surrounding the point estimate represent the 95% CI.

**Table 2. Prevalence of Infected and Infective Rate, and Seasonal Transmission Potential Estimated in Las Golondrinas, Mexico, When Using Two Methods of Parasite DNA Purification.**

## Discussion

The aim of the present study was to compare the gold standard P-C method of parasite DNA purification from pools of heads or bodies from *S. ochraceum* s.l. flies and the DNA purification method based on the paramagnetic particle. The experimental strategy followed was to increase the pool size of fly heads or bodies tested by O-150 PCR-ELISA using the genomic DNA of the Maxwell® method, thus decreasing the labour involved during the purification of DNA. The gold standard P-C method takes about 2 days for processing 20 pools of 50 heads or bodies of flies, each using considerable amounts of phenol and chloroform, which are both toxic reagents, while the Maxwell® paramagnetic particle method takes about 45 minutes to process 16 pools of 100 heads or bodies of flies each without exposure to any toxic chemicals. There was no statistical difference in the estimated transmission parameters using both DNA purification methods. Hence, the paramagnetic particle protocol could be used as an effective and reproducible alternative to the long-extraction, gold-standard protocol currently being used for testing parasite infection in pools of fly heads or bodies by the onchocerciasis elimination programs, with the advantages of avoiding considerable DNA losses, minimizing use of toxic materials and saving time. The Maxwell® paramagnetic particle method has fewer steps and manipulations, thus reducing the risk of contamination with foreign DNA and time with high reliability. The beads used in the magnetic DNA preparations are silica-coated, so the Maxwell® 16 method from Promega is not a significant departure from the conventional Whatman® plate P-C method, which also uses the glass plates as a silica matrix. This means that the method reported here from Promega is similar to the conventional method and is more automated and much faster than using phenol-chloroform. Thus, the Maxwell® 16 method rapidly purified DNA from black fly heads or bodies without losing sensitivity for detection of *Onchocerca volvulus*.

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## REFERENCES

1. Meredith, S.E. *et al.* (1991) *Onchocerca volvulus*: Application of the polymerase chain reaction to identification and strain differentiation of the parasite. *Exp. Parasitol.* **73**, 335–44.
2. Rodríguez-Pérez, M.A. *et al.* (2008) Rapid suppression of *Onchocerca volvulus* transmission in two communities of the Southern Chiapas focus, Mexico, achieved by quarterly treatments with Mectizan. *Am. J. Trop. Med. Hyg.* **79**, 239–44.
3. Rodríguez-Pérez, M.A. *et al.* (1999) Comparison of serological and parasitological assessments of *Onchocerca volvulus* transmission after 7 years of mass ivermectin treatment in Mexico. *Trop. Med. Intl. Health* **4**, 98–104.
4. Rodríguez-Pérez, M.A. *et al.* (2004) Polymerase chain reaction monitoring of transmission of *Onchocerca volvulus* in two endemic states in Mexico. *Am. J. Trop. Med. Hyg.* **70**, 38–45.
5. Katholi, C.R. *et al*. (1995) Determining the prevalence of *Onchocerca volvulus* infection in vector populations by polymerase chain reaction screening of pools of black flies. *J. Infect. Dis.* **172**, 1414–7.

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