Utilising Whatman FTA card technology to identify cell free schistosome DNA in a mouse model

Madeleine J. Rogers[1], Catherine A. Gordon[1,2], Donald P. McManus[1,2]

[1]University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, Australia

[2]QIMR Berghofer Medical Research Institute, Molecular Parasitology laboratory, Brisbane, Australia

QIMR Berghofer Medical Research Institute

THE FUTURE OF HEALTH

THE UNIVERSITY OF QUEENSLAND

The Australian Society for Parasitology Inc.

Background

- Schistosomiases is a neglected tropical disease afflicting over 240 million people, with over 700 million people at risk in 78 endemic countries.
- *S. japonicum,* known as the Asian schistosome, causes intestinal schistosomiasis, and is found in Indonesia, China, and the Philippines (Figure 1)[1]

Control and diagnosis (Figure 1)

- Complex life-cycle – complicates control
- Mass drug administration of praziquantel with limited case finding[2].
- Current microscopic based diagnostic methods lack sensitivity and specificity[3].
- More sensitive methods (DNA based detection) are inaccessible in remote endemic areas due to cost[3].

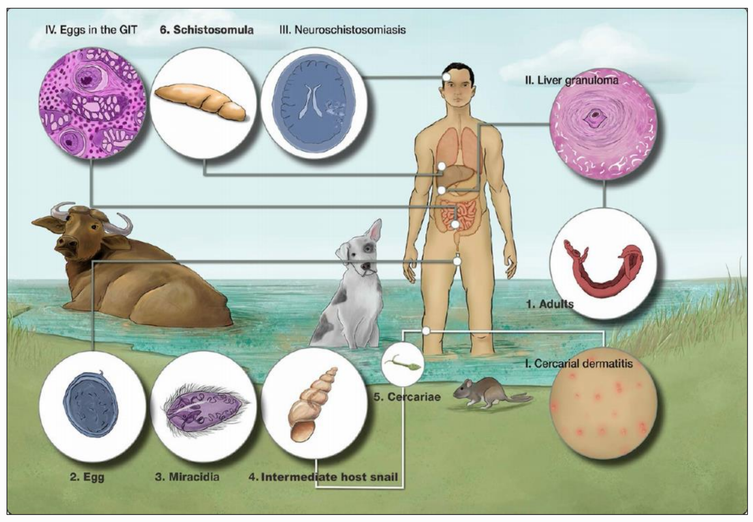

Figure 1: *Schistosoma spp.* life cycle and disease progression.

Problems with *S. japonicum* diagnosis

- Sample collection involves laborious methods that can be painful (blood and serum) and uncomfortable (faeces and urine), resulting in low compliance[4].
- Sample storage and collection is an issue due to lack of access to correct storage equipment and rapid transport protocols [5].
- Whatman FTA cards can store samples and maintain DNA integrity at room temperature for up to 10 years [6]

Aim and Hypothesis

Aims

1. To validate the use of Whatman FTA cards as a storage medium for Schistosome cfDNA in clinically relevant samples (whole blood, serum, urine, faeces, saliva)
2. To determine the diagnostic efficacy of detecting cfDNA present in urine, faeces, blood, serum, and saliva stored on Whatman FTA cards by qPCR and ddPCR

Hypothesis

1. Whatman FTA cards can preserve Schistosome cfDNA for from different clinical samples (blood, serum, urine, faeces, saliva)
2. cfDNA from FTA cards is detectable in all molecular diagnostic techniques (qPCR, ddPCR)
3. Copy number index values will be highest at day 41 post-infection in all samples types.

Methods

- 12 female adult Swiss outbread mice split into two groups (group A and group B) (Figure 2)
  - Group A: Infected with 70 *S. japonicum* Philippines strain cercariae per mouse
  - Group B: Infected with 50 *S. japonicum* Chinese strain cercariae per mouse
- Samples on FTA cards dried in laminar flow hood for 1 week
- Mice sacrificed and perfused 42 days post infection (dpi).
- Liver harvested for egg count analysis, salivary glands harvested and placed on FTA cards.
- cfDNA extracted from FTA cards for qPCR and ddPCR (Figure 4).

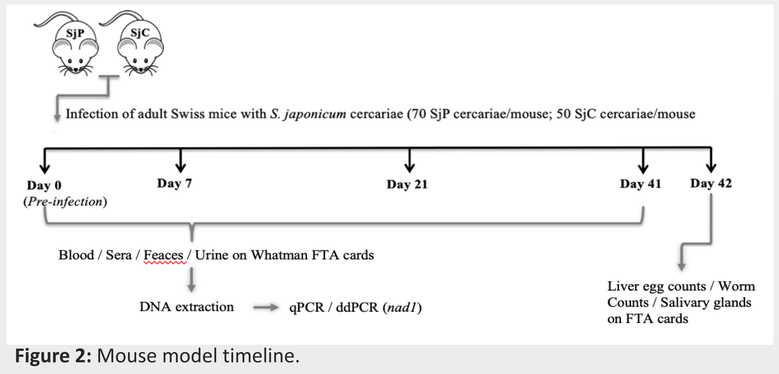

Figure 2: Mouse model timeline.

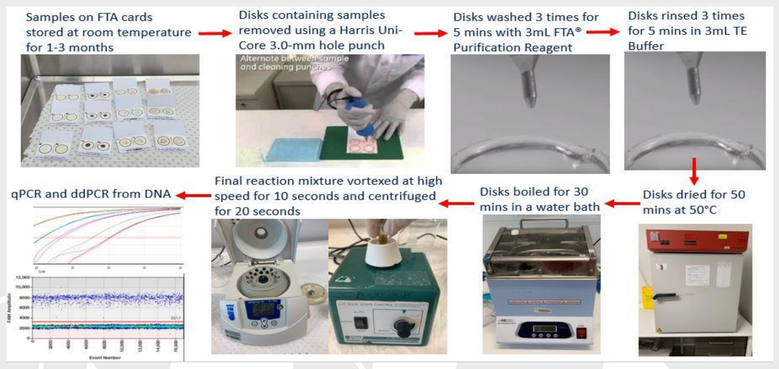

Figure 4: cfDNA extraction from FTA cards

Results

Table 1: Summary results (positive/negative) of qPCR for various sample types at different time points

qPCR		Blood		Serum		Faeces		Urine		Salivary Glands	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
S. japonicum (Philippine strain)	Day 0	0	6	0	6	0	6	0	6	-	-
	Day 7	0	6	0	6	4	2	1	5	-	-
	Day 21	1	5	0	6	3	3	2	4	-	-
	Day 41	2	4	0	6	5	1	0	6	2	4
	Total	3	21	0	24	12	12	2	22	2	4
S. japonicum (Chinese strain)	Day 0	0	0	0	6	-	-	0	6	-	-
	Day 7	2	4	3	3	2	4	0	6	-	-
	Day 21	2	4	1	5	0	6	0	6	-	-
	Day 41	1	5	2	4	3	3	0	6	0	6
	Total	5	13	6	18	5	13	0	24	0	6

- Samples considered positive if 2 or more replicates amplified on the melt curve.

Table 2: Sensitivity of qPCR for all sample types at 41 dpi.*

	SjP		SjC	
Assay	**Sensitivity (%)**	**95% CI**	**Sensitivity (%)**	**95% CI**
B_qPCR	33.33	15.81-100	16.67	2.5-100
S_qPCR	0	0	33.33	15.81-100
F_qPCR	83.33	47.82-100	50	29.24-100
U_qPCR	0	0	0	0
SG_qPCR	33.33	15.81-100	0	0

B_qPCR, Blood qPCR; S_qPCR, Serum qPCR; F_qPCR, Faeces qPCR; U_qPCR, Urine qPCR; SG_qPCR, Salivary Gland qPCR

*Liver EPG used as standard denominator

Table 3: Summary results (positive/negative) of ddPCR for various sample types at different time points

ddPCR		Blood		Serum		Faeces		Urine		Salivary Glands	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
S. japonicum (Philippine strain)	Day 0	0	6	0	6	0	6	0	6	-	-
	Day 7	6	0	4	2	6	0	6	0	-	-
	Day 21	6	0	5	1	6	0	2	4	-	-
	Day 41	6	0	4	2	6	0	6	0	3	3
	Total	18	6	13	11	24	0	14	10	3	3
S. japonicum (Chinese strain)	Day 0	0	6	0	6	-	-	0	6	-	-
	Day 7	0	6	1	5	6	0	6	0	-	-
	Day 21	0	6	0	6	6	0	5	1	-	-
	Day 41	0	6	0	6	6	0	5	1	4	2
	Total	0	24	1	23	18	0	16	8	4	2

Table 4: Sensitivity of ddPCR for all sample types at 41 dpi.*

	SjP		SjC	
Assay	**Sensitivity (%)**	**95% CI**	**Sensitivity (%)**	**95% CI**
B_ddPCR	100	54.07-100	0	0
S_ddPCR	66.7	39.76-100	0	0
F_ddPCR	100	54.07-100	100	54.07-100
U_ddPCR	100	54.07-100	83.3	47.82-100
SG_ddPCR	50	29.24-100	66.67	39.76-100

*Liver EPG used as standard denominator

Conclusion

1. This is the first study to combine Whatman FTA card technology with the molecular detection of schistosome cfDNA
2. Real-time PCR:
   - Detected schistosome cfDNA in faecal samples only
3. Digital droplet PCR:
   - Detected schistosome cfDNA in blood, urine, and faeces samples from day 7 post infection with high sensitivity
4. Demonstrated:
   - Whatman FTA cards can store schistosome cfDNA for subsequent molecular diagnosis

References: **[1]** Weerakoon, K. G., et al. (2015). "Advances in the Diagnosis of Human Schistosomiasis." Clin Microbiol Rev **28**(4): 939-967. **[2]** Guo, J., et al. (2006). "A drug-based intervention study on the importance of buffaloes for human Schistosoma japonicum infection around Poyang Lake, People's Republic of China." Am J Trop Med Hyg 74(2): 335-341. **[3]** Pillay, P., et al. (2014). "Real-time polymerase chain reaction for detection of Schistosoma DNA in small-volume urine samples reflects focal distribution of urogenital Schistosomiasis in primary school girls in KwaZulu Natal, South Africa." Am J Trop Med Hyg 90(3): 546-552 **[4]** Kato, H., Caceres, A.G., Mimori, T., Ishimaru, Y., Sayed, A.S.M., Fujita, M., Iwata, H., Uezato, H., Velez, L.N., Gomez, E.A.L., and Hashiguchi, Y. (2010) Use of FTA Cards for Direct Sampling of Patients' Lesions in the Ecological Study of Cutaneous Leishmaniasis. *Journal of clinical microbiology* **48**: 3661. **[5]** Reck, M., Tomasch, J., Deng, Z., Jarek, M., Husemann, P., and Wagner-Dobler, I. (2015) Stool metatranscriptomics: A technical guideline for mRNA stabilisation and isolation. *BMC Genomics* **16**: 494. **[6]** Harty, L.C., Garcia-Closas, M., Rothman, N., Reid, Y.A., Tucker, M.A., and Hartge, P. (2000) Collection of buccal cell DNA using treated cards. *Cancer Epidemiol Biomarkers Prev* **9**: 501-506.

qimrberghofer.edu.au