



## **Bilharzia urine test strip for the detection of *Schistosoma* antigen (CCA) in urine**

### ***SCHISTOSOMA* Circulating Cathodic Antigen**

---

**Protocol for sending July 2008  
The following batch numbers:**

<b>CCA strips batch no:</b>	<b>071219</b>
<b>CCA IDIS carbon tubes batch no:</b>	<b>071211</b>
<b>CCA IDIS buffer batch no:</b>	<b>071211</b>

**Rapid Medical Diagnostics  
South Africa  
Tel +27 825298404  
Email: neelsvan@iafrica.com**

---

---

## Schistosomiasis

Schistosomiasis, also known as Bilharzia, is one of the major parasitic diseases in tropical areas, leading to chronic ill health. The main species causing human schistosomiasis are *S. mansoni* and *S. japonicum* (causing intestinal schistosomiasis) and *S. haematobium* (causing urinary schistosomiasis). The life cycle (see figure 7) involves a fresh-water snail as an intermediate host. The infective stage to man is the cercaria, which may cause cercarial dermatitis ("swimmer's itch"). Many, in particular light infections are asymptomatic. Acute symptoms (Katayama's fever) may occur 2-8 weeks after the initial infection. Manifestations include fever, cough, abdominal pain, diarrhoea and eosinophilia. Acute schistosomiasis is frequently seen in travellers returning from a visit to endemic areas. The subsequent chronic stages of infection are the result of the formation of granulomas around eggs trapped in the intestinal wall, liver, bladder, reproductive organs, spinal cord etc. The symptoms include colicky pains, diarrhoea, sometimes with blood and mucus, hematuria. In later stages signs secondary to portal hypertension may result, such as hepatomegaly, splenomegaly and/or hematemesis. In urinary schistosomiasis lesions of kidney and urethra may be prominent. The risk of serious disease is greatest in heavy infections, when many worms are present in the host.

Diagnosis of schistosomiasis is usually performed by parasitological (microscopically detection of eggs) or immunological methods (antibody and antigen detection). The **Bilharzia Urine CCA** strip detects the schistosome Circulating Cathodic Antigen (CCA, present in all *Schistosoma* species, including animal species) in the urine of infected individuals. The concentration of CCA is generally dependent on the intensity of the infection, ranging approx. from 1 µg/ml urine (very high infection) to background levels of 2 ng/ml. Successful treatment may reduce the level of CCA in urine to background levels in a few days. However, if exposure patterns are not changed, reinfection may occur rapidly (resulting in positive levels in 6 to 10 weeks after initial successful chemotherapy).

### Schistosomiasis species and sensitivity

For *S. mansoni* and *S. japonicum* infections, CCA levels in urine are high and therefore the CCA strip is very sensitive for *S. mansoni* and *S. japonicum* infections.

However, for *S. haematobium* infections, CCA levels in urine are much lower. They usually are close to the cut-off point of the CCA strips (standard 100). Therefore, please note that the CCA strip is not very sensitive for *S. haematobium* infections in low transmission areas.

### Cross-reactivity

A previous study showed that cases of urinary tract infection may lead to false positive results on CCA strips due to cross-reactivity of the antigen.

---

## Contents

- CCA strips batch 071219 (5 x 100 pieces per container incl. 1 dry pack),
- CCA IDIS carbon tubes batch 071211 (5 x 96 + 1 x 20 tubes refill racks)

### **Store at room temperature at a DRY place upon arrival**

- CCA IDIS buffer batch 071211 (6 x tube lyophilized, add 10 ml milliQ water just before use)
- rack (2 x, empty, to be filled with CCA carbon tubes, re-usable)
- QC standards batch 080114 (0, 10, 100, 1000, 10.000)

### **store at -20°C upon arrival**

## Storage

All components must be stored at room temperature (18°C) in a DRY place and expire 18 months after production date (see labels).

CCA IDIS buffer is provided in lyophilized state, and should be stored at room temperature before use.

After 10 ml distilled water is added, store at +4°C and use within 7 days.

QC standards and AWA-TCA standard: **store at -20°C**

## Precautions

Human urine must be handled as potential infectious material.

Always use the buffer that comes with the tests. Do not mix buffers or tubes from other lots.

## Disclaimer

The entire risk as to the performance of these products is assumed by the purchaser. Bilharzia Rapid Diagnostics shall not be liable for indirect, special or consequential damages of any kind resulting from use of these products.

---

## Test principle

The principle of the test is an antigen-capture assay. By mixing the sample and the carbon-conjugate containing buffer, the *Schistosoma* CCA is bound to the antibody-carbon particles. Lateral flow transports the CCA-carbon complex through the strip, where it – if CCA is present - will bind to the anti-CCA antibody at the testline, showing a black carbon precipitation. Excess carbon conjugate is caught by the control line.

## Test Procedure

### 1. Preparation:

#### Collect:

- CCA IDIS Carbon tubes, containing dried antibody carbon conjugate  
One carbon tube can only be used once, for one sample.
- CCA strips, one strip can only be used once, for one sample.  
The strips contain 2 sides, a sample pad side (shorter pad, ca. 1 cm) and an adsorbent pad side (longer pad, ca. 2 cm) as shown in figure 4B.
- CCA IDIS buffer (lyophilized in tube, 1 tube is for 5 ml buffer)
- Urine samples to be tested
- Standards
- Pipettes (for 75 µl and 25 µl) and pipette tips
- Distilled water

#### Buffer preparation:

CCA buffer: Add 10 ml of distilled water (e.g. MilliQ) to 1 tube of lyophilized CCA buffer. Make sure the suspension is homogenous.

You will need 75 µl CCA buffer per sample (1 tube is sufficient for 100 samples)

Write the preparation date on the tube. Use within 7 days and store at +4°C.

#### Standards:

Standard 100 is the cut-off sample. It is highly recommended to run this sample every time CCA strips are used.

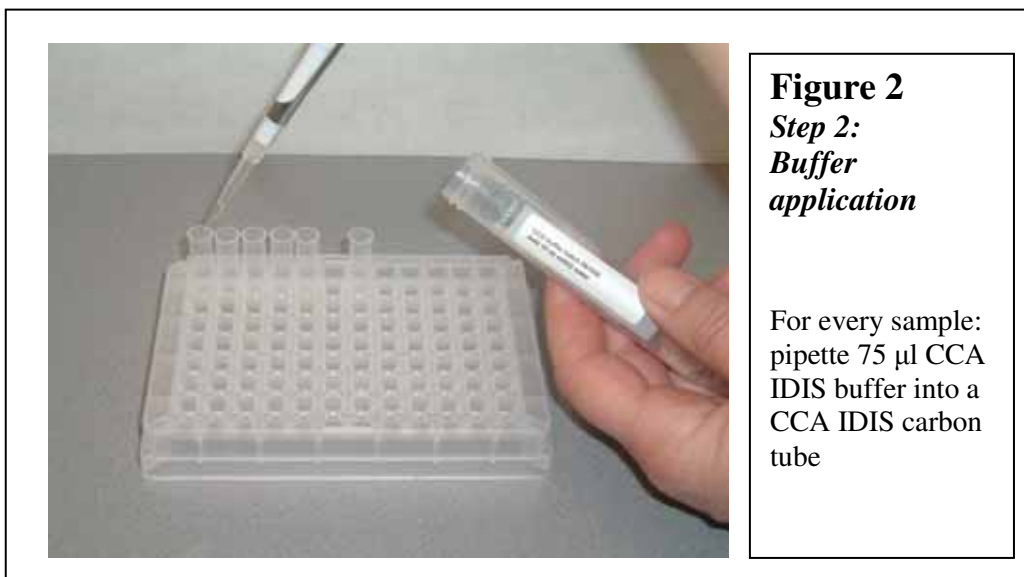
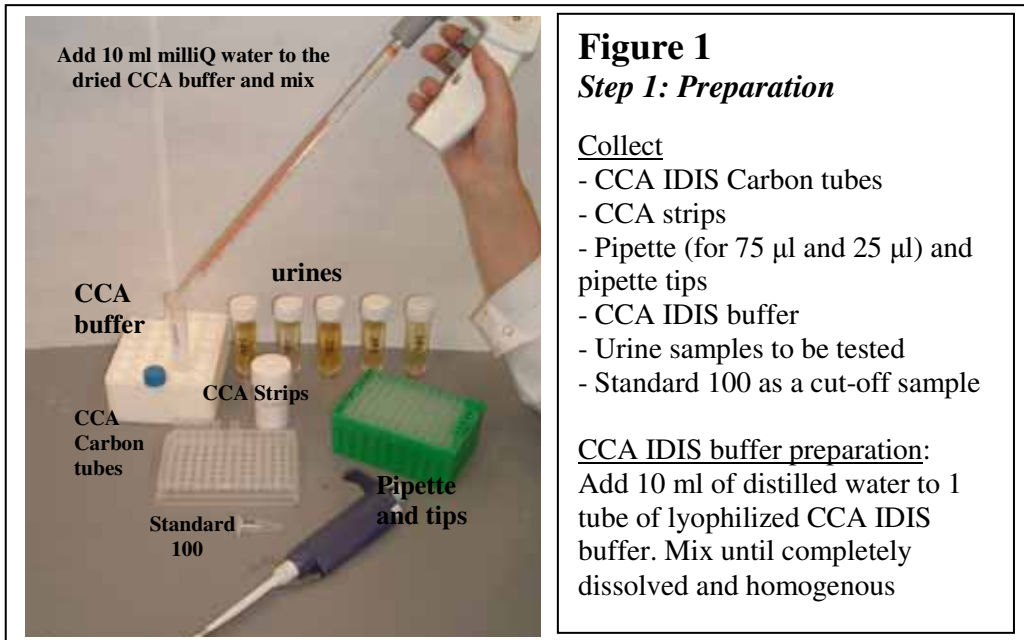
The CCA strip must be considered negative when the testline is weaker than standard 100, and positive when the testline is stronger than standard 100.

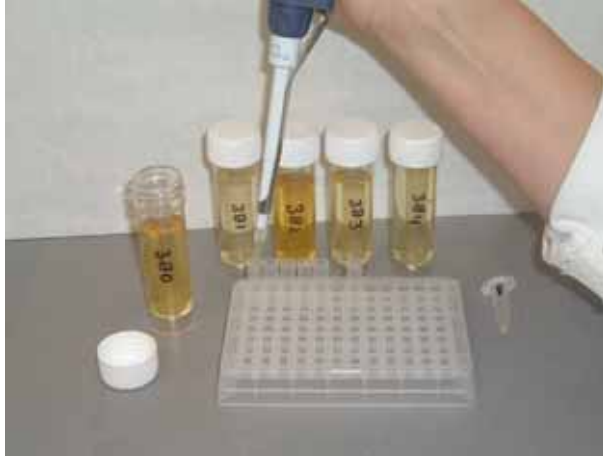
It is highly recommended to run all standards (10000, 1000, 100, 10 and 0) as a quality control with every 100 urine samples tested.

See figures for a visual representation of the test procedure.

In the photographs five urine samples are tested and standard 100 is run as a cut-off sample.

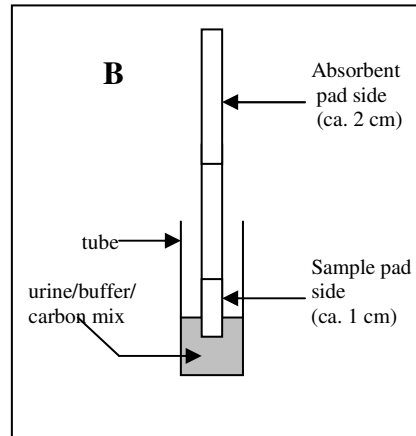
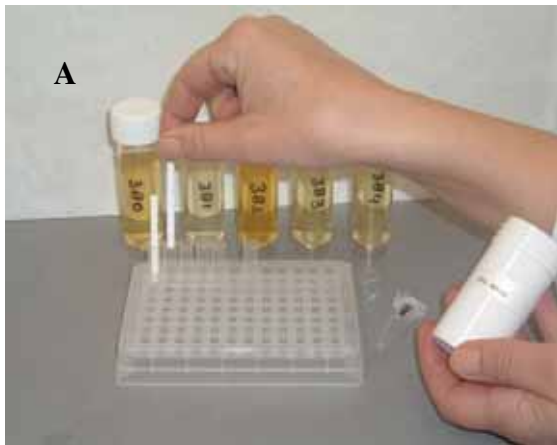
2. For every sample: pipette 75 µl CCA IDIS buffer into a CCA IDIS carbon tube (figure 2)
3. Pipette 25 µl urine (or standard) into the buffer and mix by pipetting up and down for at least 5 times. Make sure the suspension is homogenous (figure 3).
4. ATTENTION: there are two sides on the CCA strips: a sample-pad side (ca. 1 cm glasfibre material) and an absorbent-pad side (ca. 2 cm tissue material). See figure 4B for the right orientation of the strip and insert the CCA strips with the sample-pad down into the 100 µl mix of urine/buffer/carbon (figure 4).
5. Incubate for 40 minutes at room temperature, and read the results against the standards as described in step 6.





**Figure 3**  
**Step 3:**  
**Sample application**

Pipette 25  $\mu$ l urine into the buffer and mix by pipetting up and down for at least 5 times. Make sure the suspension is homogenous



**Figure 4**  
**Step 4: Insertion (A) and orientation (B) of the strips**

Insert the CCA strips with the samplepad side (shorter side) down into the 100  $\mu$ l mix of urine / buffer / carbon



**Figure 5:**  
**Step 5:**  
**Incubation**

Incubate for 40 minutes at room temperature, and read the results against the standards

## 6. Interpretation of results

### Positive/negative discrimination

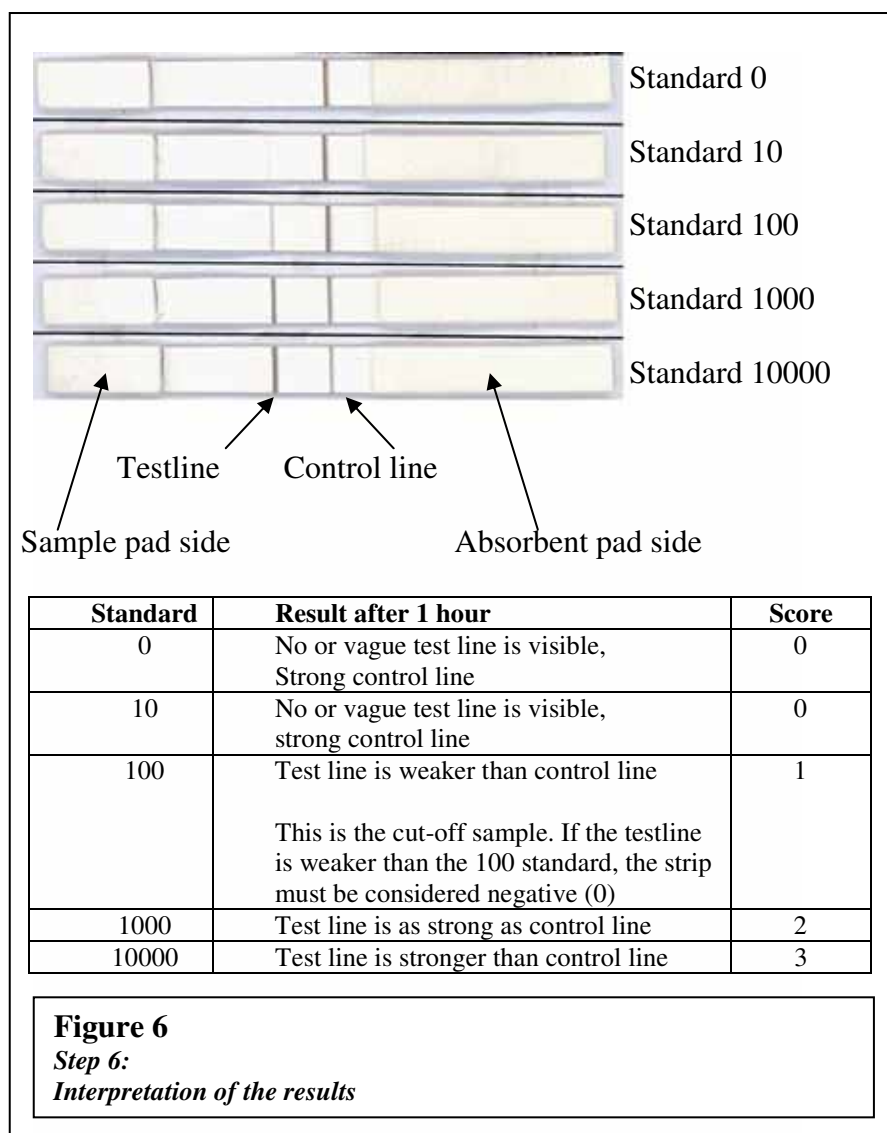
After 40 minutes incubation, the CCA strip must be read against the cut-off sample standard 100. The test must be considered negative when the testline is not visible or weaker than standard 100, and positive when the testline is visible or stronger than standard 100.

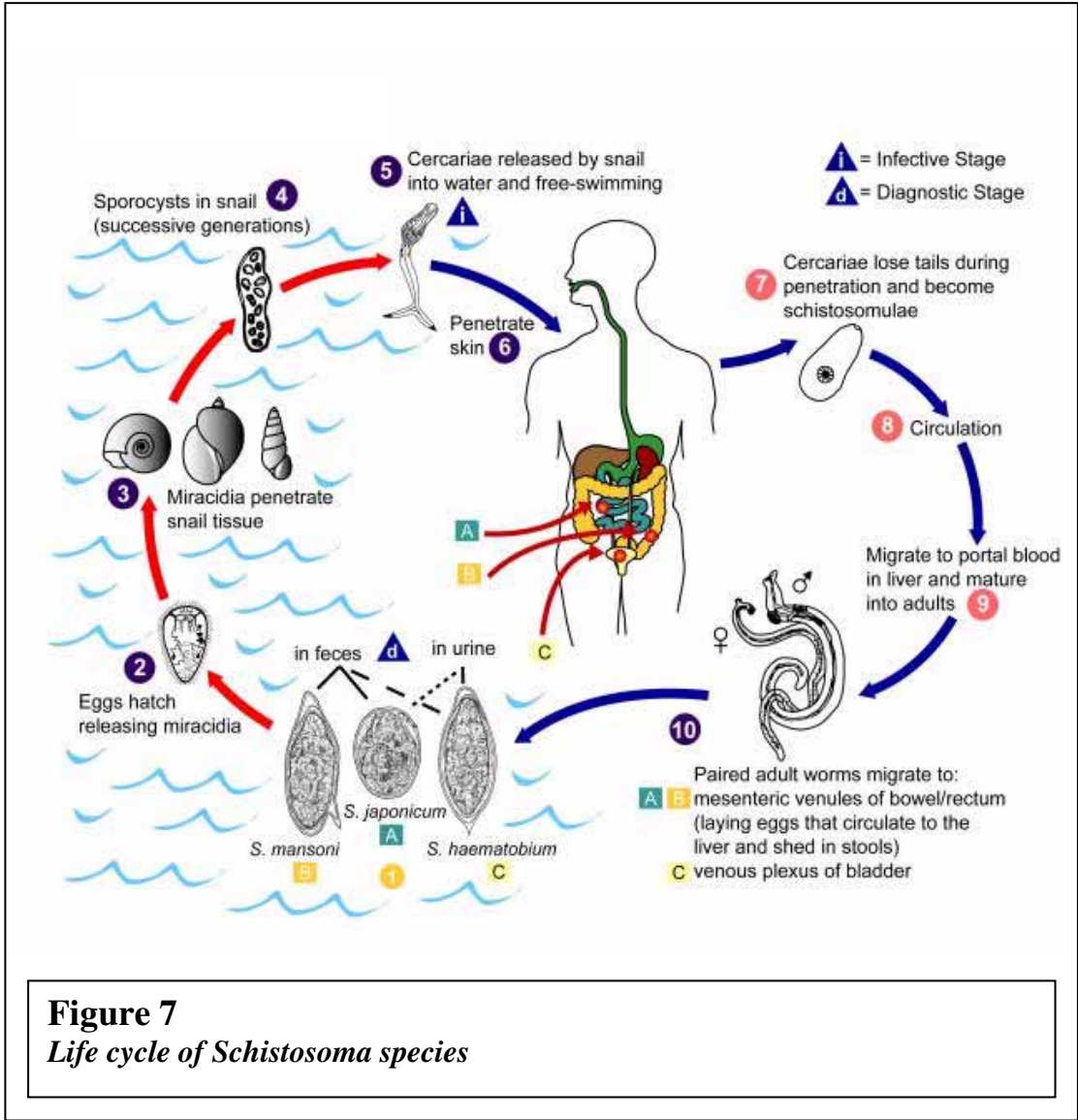
It is highly recommended to run cut-off sample standard 100 every time CCA strips are used.

### Semi-quantitative reading

After 40 minutes incubation, the CCA strips must be read and scored against all standards as described in figure 6.

It is highly recommended to run all standards 10000, 1000, 100, 10 and 0 within every 100 urine samples as a quality control. Eventually, after drying, the strips can be fixed to a paper and stored as a permanent record or to be read afterwards by a second reader.





**Figure 7**  
*Life cycle of Schistosoma species*