



ImmunoComb® II

HIV 1 & 2 CombFirm



Code: 60434002

Format: 3 x 6 tests

The ImmunoComb® II HIV 1 & 2 CombFirm Kit is a confirmation test. The test allows to confirm an initial HIV reactive human serum or plasma specimen. Eighteen tests may be performed with one kit.

Introduction

The Human Immunodeficiency Virus (HIV) is a retrovirus, identified in 1983 as the etiologic agent of the Acquired Immunodeficiency Syndrome (AIDS). Two types, HIV-1 and HIV-2, exhibiting different prognosis and transmission rates, have been distinguished.

The major routes of HIV transmission are sexual contact, contamination by blood or blood products, and mother-to-newborn transmission. HIV predominantly infects CD4 lymphocytes, which play a key role in the human immune system. The progressive decrease of the CD4 level during development of the disease leads to an immunocompromised condition, facilitating opportunistic infections with fatal consequences.

The HIV virus consists of a genomic RNA molecule associated with a reverse transcriptase, protected by a capsid and an envelope. The virus envelope is the major target for humoral antibody response. Consequently, highly sensitive HIV screening tests are primarily based on the detection of antibodies to antigens derived from envelope (*env*) components.

To distinguish true from false reactions, a positive result by an HIV screening assay should be retested and confirmed by a test of higher specificity. Serological confirmation of an HIV infection, commonly performed using a Western blot assay, is based on the differential detection of antibodies directed against HIV antigens. The ImmunoComb® II HIV 1 & 2 CombFirm test employs five distinct recombinant and synthetic HIV antigens for the rapid serological determination of the HIV antibody profile. Two antigens are *pol* and *gag*-derived proteins reactive with both HIV types, whereas three antigens are *env*-derived peptides or proteins, specific for either HIV-1 or HIV-2. The antigens are separately positioned at fixed concentrations and free of tissue antigens, which may contaminate antigen preparations from the viral lysates commonly used in Western blots.

The test results of the ImmunoComb® II HIV 1 & 2 CombFirm test are comparable to those obtained by Western blot. Instead of the necessity to run two time-consuming blots, reactivity for antibodies to HIV types 1 or 2 may be rapidly confirmed in a single simple test.

Principle of the Test

The ImmunoComb® II HIV 1 & 2 CombFirm test is an indirect solid-phase enzyme immunoassay (EIA). The solid phase is a card with 12 projections ("teeth"). Each card has 6 pairs of teeth, with six antigen spots per pair (3 spots on each tooth). The left tooth of each pair carries an upper spot sensitized with human immunoglobulin (Internal Control),

and the two protein markers p24 (*gag*) and p31 (*pol*). The right tooth has three *env*-derived protein spots gp41, gp120 and gp36.

The Developing Plate has 6 rows (A-F), each row containing a reagent solution ready for use at a different step in the assay. The test is performed stepwise, by moving the Card from row to row, with incubation at each step.

To start the test, serum or plasma specimens are added to the diluent in the wells of row A of the Developing Plate. The Card is then inserted in the wells of row A. Anti-HIV antibodies, if present in the specimens, will specifically bind to the HIV antigens on the teeth of the Card (Figure 1). Unbound components are washed away in row B. In row C, the anti-HIV IgG captured on the teeth, and the human immunoglobulin on the upper spots (Internal Control), will react with anti-human IgG antibodies labeled with alkaline phosphatase (AP). In the next two rows, unbound components are removed by washing. In row F, the bound alkaline phosphatase will react with chromogenic components. The results are visible as gray-blue spots on the surface of the teeth of the Card.

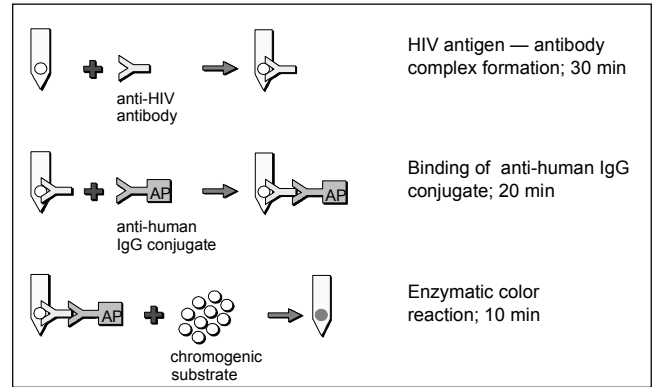


Figure 1. Principle of the Test

The kit includes a Positive Control (antibodies to HIV-1 and HIV-2) and a Negative Control to be included in each assay run. Upon completion of the test, the pair of teeth used with the Positive Control should show all 6 gray-blue spots, and that used with the Negative Control should show solely the Internal Control spot. The Internal Control spot should also appear on all other teeth, to confirm that the kit functions properly and that the test was performed correctly.

Kit Contents

Cards

The kit contains 3 plastic Cards. Each Card has 6 pairs of teeth, one pair for each test (Figure 2). Both teeth of a pair carry identical numbers. Each pair of teeth is sensitized with six reactive areas:

Position	On left tooth	On right tooth
Upper	Human immunoglobulin (Internal Control)	Recombinant HIV-1 <i>env</i> -derived glycoprotein gp120
Middle	Recombinant <i>gag</i> -derived core protein p24 (recognized by antibodies to HIV-1 & HIV-2)	HIV-1 transmembrane <i>env</i> - glycoprotein gp41-derived synthetic peptide
Lower	Recombinant <i>pol</i> -derived protein p31 (recognized by antibodies to HIV-1 & HIV-2)	HIV-2 transmembrane <i>env</i> glycoprotein gp36-derived synthetic peptide

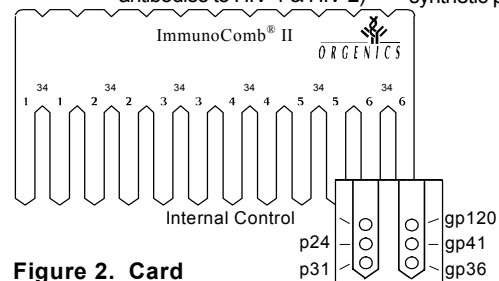


Figure 2. Card

The Cards are provided in aluminum pouches containing a desiccant bag.

Developing Plates

The kit contains 3 Developing Plates, covered by aluminum foil. Each Developing Plate (Figure 3) contains all reagents needed for the test. The Developing Plate consists of 6 rows (A-F). Row A is divided into 6 wells, whereas the other rows are divided into 12 wells each. The contents of each row are as follows:

- Row A specimen diluent
- Row B washing solution
- Row C alkaline phosphatase-labeled goat anti-human IgG antibodies
- Row D washing solution
- Row E washing solution
- Row F chromogenic substrate solution containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT)

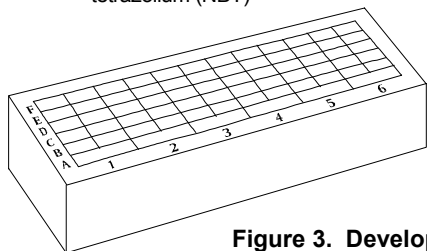


Figure 3. Developing Plate

Positive Control — 1 vial (red-colored cap) of 0.4 mL diluted human plasma positive for anti-HIV-1 and anti-HIV-2 antibodies, inactivated by addition of b-propiolactone and by heat treatment.

Negative Control — 1 vial (green-colored cap) of 0.4 mL diluted heat-inactivated human plasma, negative for antibodies to HIV.

Perforator — for perforation of the aluminum foil, covering the wells of the Developing Plate.

Safety and Precautions

- This kit is for *in vitro* diagnostic use only.
- Handle the Positive Control as if potentially infectious even though it has been inactivated.
- All other human source materials used in the preparation of the controls were tested and found to be non-reactive for hepatitis B surface antigen, for antibodies to hepatitis C virus and for antibodies to HIV. Since no test method can give complete assurance of the absence of viral contamination, all reference solutions and all human specimens should be handled as potentially infectious.
- Wear surgical gloves and laboratory clothing. Follow accepted laboratory procedures for working with human serum or plasma.
- Do not pipette by mouth.
- Dispose of all specimens, used Cards*, Developing Plates, and other materials used with the kit as biohazardous waste.
- Do not mix reagents from different lots.
- Do not use the kit after expiry date.

Storage of the Kit

Store the kit in its original box at 2°–8°C. Under these conditions, the kit will remain stable until the expiry date on the label. Do not freeze the kit.

Handling of Specimens

You may test either serum or plasma specimens. These should be totally separated from red blood cells by at least one centrifugation step. Specimens may be stored for 72 hours at 2° to 8°C before testing. To store for more than 72 hours, freeze specimens at –20°C or colder. After serum specimens have thawed, centrifuge them. Test the supernatant. Avoid repeated freezing and thawing.

Test Procedure

Equipment Needed

- Precision pipette with disposable tips for dispensing 50 µl
- Scissors
- Laboratory timer or watch

Preparing the Test

Bring all components, developing plates, cards, reagents and specimens to room temperature and perform the test at room temperature (22°–26°C).

Preparing the Developing Plate

1. Incubate the Developing Plate in an incubator at 37°C for 30 minutes; or leave at room temperature (22°–26°C) for 3 hours.
2. Cover the work table with absorbent tissue to be discarded as biohazardous waste at the end of the test.
3. Mix the reagents by shaking the Developing Plate.

Note: Do not remove the foil cover of the Developing Plate. Break the foil cover by using the disposable tip of the pipette or the perforator, only when instructed to do so by the Test Instructions.

Preparing the Card

Caution: To ensure proper functioning of the test, do not touch the teeth of the Card.

1. Tear the aluminum pouch of the Card at the notched edge. Remove the Card.
2. You may use the entire Card and Developing Plate or only a part. To use part of a Card:
 - a. Determine how many pairs of teeth you need for testing the specimens and controls. You need one pair for each test. Each pair of teeth displays the code number "34" of the kit, to enable identification of detached teeth.
 - b. Bend and break the Card vertically or cut with scissors (see Figure 4) to detach the required number of teeth **pairs** (No. of tests including 2 controls). Do not separate paired teeth.
 - c. Return the unused portion of the Card to the aluminum pouch (with desiccant bag). **Close pouch tightly**, e.g. with a paper clip, to maintain dryness. Store the Card in the original kit box at 2°–8°C for later use.

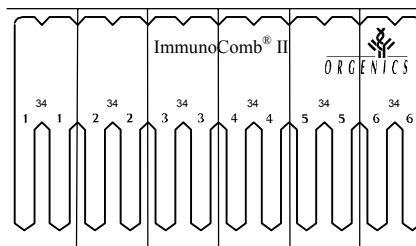


Figure 4. Breaking the Card

Test Instructions

Antigen–Antibody Reaction (Row A of the Developing Plate)

1. Pipette 50 µl of specimen. Perforate the foil cover of one well in row A of the Developing Plate with the pipette tip or perforator and dispense the specimen at the bottom of the well. **Mix** by repeatedly refilling and ejecting the solution. Discard pipette tip.
2. Repeat step 1 for the other specimens, including one Positive and one Negative Control supplied with the kit. Use a new well in row A and change pipette tip for each specimen or control.
3. a. Insert the Card (**printed** side facing you) into the wells of row A containing specimens and controls.

Mix: Withdraw and insert the Card in the wells several times.
- b. Leave the Card in row A for exactly 30 minutes. Set the timer. Near the end of 30 minutes, perforate the foil of row B using the perforator. Do not open more wells than needed.
- c. At the end of 30 minutes, take the Card out of row A.

Absorb adhering liquid from the **pointed tips** of the teeth on clean absorbent paper. Do not touch the front surface of the teeth.

First Wash (Row B)

4. Insert the Card into the wells of row B. **Agitate:** Vigorously withdraw and insert the Card in the wells for at least 10 seconds to achieve proper washing. Repeat agitation several times during the course of 2 minutes; meanwhile perforate the foil of row C. After 2 minutes, withdraw the Card and **absorb adhering liquid** as in step 3c.

Binding of Conjugate (Row C)

5. Insert the Card into the wells of row C. **Mix** as in step 3a. Set the timer for 20 minutes. Perforate the foil of row D. After 20 minutes, withdraw the Card and **absorb adhering liquid**.

Second Wash (Row D)

6. Insert the Card into the wells of row D. Repeatedly **agitate** during 2 minutes, as in step 4. Meanwhile perforate the foil of row E. After 2 minutes, withdraw the Card and **absorb adhering liquid**.

Third Wash (Row E)

7. Insert the Card into the wells of row E. Repeatedly **agitate** during 2 minutes. Meanwhile perforate the foil of row F. After 2 minutes, withdraw the Card and **absorb adhering liquid**.

Color Reaction (Row F)

8. Insert the Card into the wells of row F. **Mix.** Set the timer for 10 minutes. After 10 minutes, withdraw the Card.

Stop Reaction (Row E)

9. Insert the Card again into row E. After 1 minute, withdraw the Card and allow it to dry in the air.

Waste Disposal

Dispose of used Developing Plates, pipette tips, absorbent paper, and gloves as biohazardous waste.

*Unless stored for documentation

Storing Unused Part of Kit

Developing Plate

If you have not used all the wells of the Developing Plate, you may store it for future use:

- Seal used wells with wide adhesive tape so that nothing can spill out of the wells, even if Developing Plate is tipped over.

Other Kit Materials

- Return remaining Developing Plate(s), Card(s), perforator, controls, and instructions to the original kit box. Store at 2°–8°C.

Test Results

Validation

In order to confirm that the test functions properly and to demonstrate that the results are valid, the following three conditions must be fulfilled (see Figure 5):

- The **Positive Control** must produce **six** gray-blue colored spots on the relevant pair of Card teeth.
- The **Negative Control** must produce an **upper** spot (Internal Control) on the **left** tooth of the pair and no other spots.
- Each **specimen tested** must produce an **upper** spot (Internal Control) on the left tooth of the pair.

If any of the three conditions are not fulfilled, the results are invalid, and the specimens and controls should be retested.

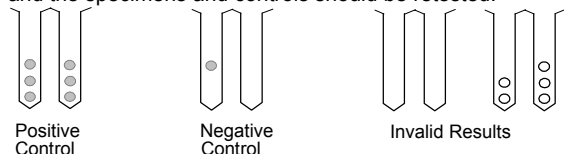


Figure 5. Test Validation

Interpretation of the Results

The **sole appearance** of the **Internal Control** indicates that the corresponding specimen is **negative** for antibodies to HIV-1 or HIV-2.

A specimen yielding only **one** isolated circular, colored HIV antigen spot, or **both** the p24 and p31 spots without a spot on the right tooth, is considered **indeterminate**.

A specimen yielding a minimum of **two** circular, colored HIV antigen spots, including one representing gp41 or gp36 (see Figure 2), is defined as **HIV-positive**. In these cases *differentiation* between HIV-1 and HIV-2 may be obtained as follows:

- Absence of a spot for **gp36** and presence of **gp41** and/or **gp120** indicates that the specimen is positive for antibodies to HIV-1.
- Presence of a spot for **gp36** indicates that the specimen is positive for antibodies to HIV-2.

The most prevalent patterns of spots and their interpretation are summarized in Table 1.

Table 1. Interpretation of the results

Pattern of HIV spots present on		Interpretation
Left tooth	Right tooth	Positive for
p24 and/or p31	gp41	HIV-1
No spot	gp41 and gp120	
p24 and/or p31	gp36 only or with gp41	HIV-2
p24 and/or p31	gp36 and gp41 and gp120	HIV-2 only, or Coinfection with HIV-1
p24 and/or p31	No spot or gp120	Indeterminate
No spot	gp41 or gp120 and/or gp36	
No spot	No spot	Negative

Important:

- Any faintly colored spot on the teeth must be interpreted as reactive.
- In case of an **indeterminate** result, collect a new blood sample and repeat the test.

Documentation of Results

As the color developed on the Card is stable, the Cards may be stored for documentation.

Limitations

The ImmunoComb® II HIV 1 & 2 CombFirm is a supplementary assay aimed at confirming the presence of antibodies to HIV antigens in initially reactive specimens. In the absence of uniformly accepted criteria for the interpretation of HIV antibody profiles, the above-described interpretation should be considered as a recommended guideline. Yet, reactivity for antibodies to HIV-1/HIV-2 must not be considered a sole criterion for the diagnosis of Acquired Immunodeficiency Syndrome (AIDS) or of infection with HIV. Since the production of antibodies may be delayed following initial exposure to HIV, or be suppressed due to the immunocompromised condition of

a patient, a negative result with this test must not be considered conclusive evidence that the patient has not been exposed to or infected by HIV.

Performance Characteristics*

A. Multicenter Study

A multicenter study was carried out on 559 specimens including 154 HIV-negative individuals, 318 HIV-1 positive patients, 35 patients that were positive by ELISA and indeterminate (Ind.) for HIV-1 by Western blot assay (WB), as well as 52 individuals that were positive by ELISA and negative by WB. The negative population included pregnant women, people with autoimmune diseases and with hepatitis infection. Results are detailed in Table 2.

Table 2. Multicenter study^a

HIV-1 Status		ImmunoComb® II HIV 1&2 CombFirm		
ELISA	WB	Negative	HIV-1 Positive	Ind.
Negative	Negative	150	0	4
Positive	Positive	2	305	12
	Ind.	20 ^b	6	9
	Negative	44	1	7

^a Preliminary results.

^b Including six WB displaying p17 or p55 only.

The following performance characteristics were calculated, including only unequivocally negative or positive results:

- Sensitivity — 99.7%
- Specificity — 100%

B. Specimens from Patients of African Origin

A study was carried out on specimens from individuals of African origin. The population consisted of 48 HIV-negative individuals, 51 patients infected with HIV-1, 84 infected with HIV-2, and 9 coinfecting with both HIV-1 and HIV-2. Results are detailed in Table 3.

Table 3. Specimens of African origin

HIV Status ^a		ImmunoComb® II HIV 1&2 CombFirm				
Negative		Neg.	HIV 1	HIV 2	HIV 1+2	Ind.
		48	0	0	0	0
Positive	HIV 1	0	51	0	0	0
	HIV 2	0	0	70	10	4 ^b
	HIV1 + 2	0	0	0	9	0

^a By ELISA and WB

^b Reactive with gp36 only


The data, excluding indeterminate results, show 100% sensitivity and specificity for HIV. All positive HIV-1 specimens, including 5 with subtype O, were correctly identified. Among the HIV-2 positive specimen 10 showed crossreactivity against gp120, resulting in an overall differential specificity of 93.8%

* Detailed data available on request.

















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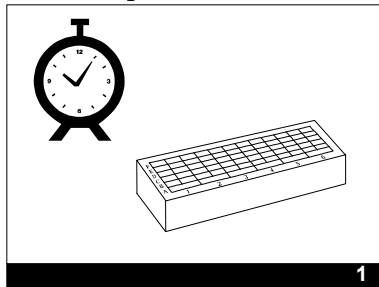
Sarnagadharan MG, Veronese F, Lee S, Gallo RC. 1985. Immunological properties of HTLV-III antigens recognized by sera of patients with AIDS and AIDS-related complex and of asymptomatic carriers of HTLV-III infection. *Cancer Res* 45:4574s-4577s.

<p>Manufacturer:</p>  <p>ORGENICS</p> <p>Orgenics Ltd., part of the Inverness Medical Innovations Group. P.O.B 360 Yavne 70650, Israel Tel: ++ 972 8 942 92 01 Fax: ++ 972 8 943 87 58</p>	<p>Authorised Representative in EU: Orgenics France S.A. 19, rue Lambrechts 92400 Courbevoie, France Tel: +33-1 41 99 92 90 Fax: +33-1 41 99 92 95</p> <p>Version: 60434002/E10/OR (01/2007)</p>
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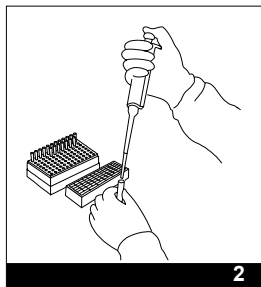
Symbols Legend

	ImmunoComb® Card
	Developing Plate
	Positive Control
	Negative Control
	Perforator
	Consult Instructions for Use
	Caution, consult accompanying documents
	In Vitro Diagnostic Medical Device
	Temperature limitation
	Contains sufficient for n tests
	Manufacturer
	Authorized Representative in the European Community
	Catalogue number
	Batch code
	Use by
	Serial number

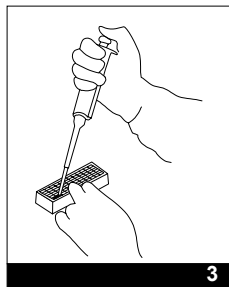
Summary of Main Test Procedures



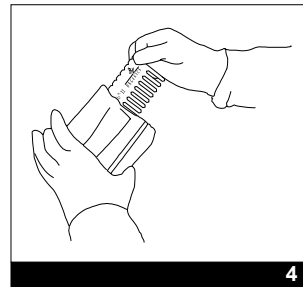
1
Preincubate the Developing Plate:
3 hrs. at room temperature,
or 30 min. at 37°C



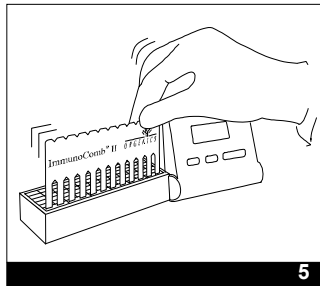
2
Draw specimens and
controls



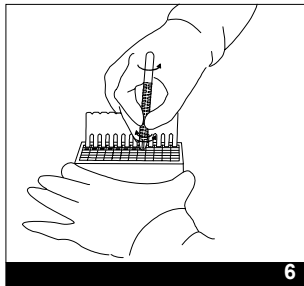
3
Add specimens and
controls to row A.
Mix



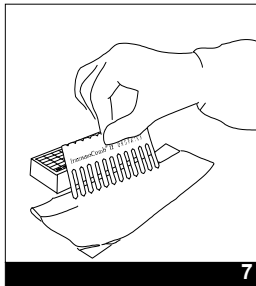
4
Remove Card from pouch



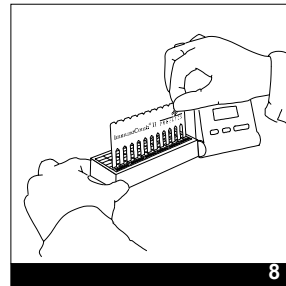
5
Insert Card in row A and mix.
Incubate



6
Open row B

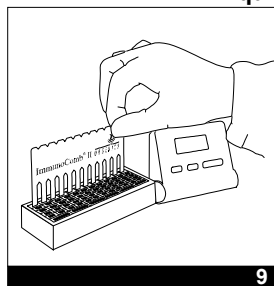


7
Absorb adhering
liquid from teeth

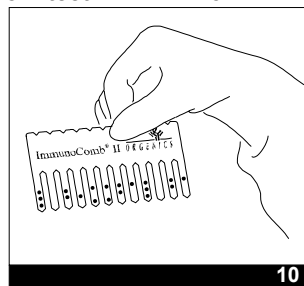


8
Insert Card and agitate in
row B. Incubate

After mixing/agitating &
incubating in rows C, D and E...



9
Color reaction in row F



10
Results

Summary of the Test Procedure

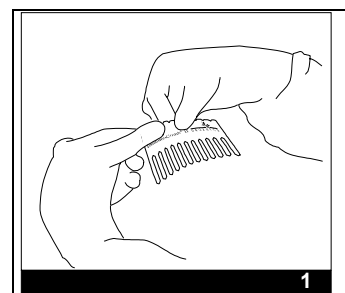
The abbreviated instructions below are for experienced users of the ImmunoComb® II HIV 1 & 2 CombFirm Kit.

(For detailed instructions please refer to complete text)

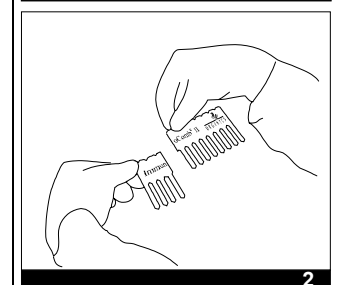
1. Bring all reagents and specimens to room temperature and perform the test at room temperature.
2. Dispense 50 µl of each specimen and control into separate wells of row A of the Developing Plate and mix.
3. Insert Card in row A and continue as described in Table 1:

Table 1. Summary of test procedure

Step	Row	Proceed as follows
Antigen-antibody reaction	A	Mix; incubate 30 minutes; absorb.
Wash	B	Agitate; incubate 2 minutes; absorb.
Binding of conjugate	C	Mix; incubate 20; absorb.
Wash	D	Agitate; incubate 2 minutes; absorb.
Wash	E	Agitate; incubate 2 minutes; absorb.
Color reaction	F	Mix; incubate 10 minutes.
Stop reaction	E	Incubate 1 minute; dry in air.



1



2

**Bending and breaking the
Card**