

# LABELING

## **The Heartsbreath Test: Product Labeling for Physicians**

**Humanitarian Device authorized by Federal law for use as an aid in the diagnosis of grade 3 heart transplant rejection in first year heart transplant recipients. The effectiveness of this device for this use has not been demonstrated.**

**Device description:** The Heartsbreath test is a non-invasive breath test for markers of oxidative stress which may predict the probability of grade 3 rejection in heart transplant recipients who received their transplants in the preceding year. It consists of:

- A breath collection apparatus for collection of volatile organic compounds in alveolar breath onto a sorbent trap, as well as for the collection of a separate sample of room air.
- Analysis of the volatile organic compounds in alveolar breath and room air by gas chromatography and mass spectroscopy.
- Interpretation of the volatile organic compounds with a proprietary algorithm in order to predict the probability of grade 3 heart transplant rejection.

**Intended use/ Indications for use:** The Heartsbreath test is indicated for use as an aid in the diagnosis of grade 3 heart transplant rejection in patients who have received heart transplants within the preceding year. The Heartsbreath test is intended to be used an adjunct to, and not as a substitute for, endomyocardial biopsy. The use of the device is limited to patients who have had endomyocardial biopsy within the previous month.

### **Contraindications:**

There are no known risks to breathing into the Heartsbreath instrument.

### **Warnings and precautions:**

- Every patient should be given a fresh new valved mouthpiece unit before the breath collection apparatus is used, in order to avoid the risk of cross infection between patients.
- Heartsbreath is not a substitute for endomyocardial biopsy – both tests should be used in combination to detect grade 3 heart transplant rejection.
- The Heartsbreath test result should be compared to biopsy performed within the previous month.
- The Heartsbreath test should not be used for patients who have received a heart transplant more than one year ago.
- The Heartsbreath test should not be used for patients who have grade 4 transplant rejection because Heartsbreath has not been evaluated in these patients.

**Potential adverse events:**

- There is a risk of cross infection if a fresh new valved mouthpiece unit is not used in the breath collection apparatus for each patient.
- The Heartsbreath test could yield a false positive or false negative result, and result in inappropriate medical management.

**Clinical information:** The Heartsbreath test employs a set of breath markers of oxidative stress<sup>1</sup>; it was evaluated in a multicenter clinical study supported by the National Heart Lung and Blood Institute. The study was entitled: Heart Allograft Rejection: Detection with Breath Alkanes in Low Levels (the HARDBALL study).<sup>2, 3</sup>

**Background:** The original clinical study evaluated a new marker of heart transplant rejection, the breath methylated alkane contour (BMAC). Rejection appears to be accompanied by oxidative stress which degrades membrane polyunsaturated fatty acids, evolving alkanes and methylalkanes which are excreted in the breath as volatile organic compounds (VOCs).

**Methods:** 1061 breath VOC samples were collected from 539 heart transplant recipients prior to scheduled endomyocardial biopsy. Breath VOCs were analyzed by gas chromatography and mass spectroscopy, and the BMAC was derived from the abundance of C4-C20 alkanes and monomethylalkanes. The gold standard of rejection was the concordant set of International Society for Heart and Lung Transplantation (ISHLT) grades in biopsies read by two cardiac pathologists.

**Results of Concordant Biopsies (Two Cardiac Pathologists):**

<b>Severity of rejection</b>	<b>ISHLT grade<sup>4</sup></b>	<b>Number of patients</b>	<b>Percentage of total</b>
<b>None</b>	0	645	60.8%
<b>Mild</b>	1A	197	18.6%
	1B	84	7.9%
<b>Moderate</b>	2	93	8.8%
<b>Severe</b>	3A	42	4.0%

No patients with grade 3B or grade 4 rejection were observed. A combination of 9 VOCs in the BMAC assigned the probability of grade 3 rejection. Comparisons of grade 3 rejection assigned respectively by the site pathologist (usually a general pathologist) readings of the endomyocardial biopsies and the breath test are shown below (TP = true positives, TN = true negatives, FP = false positives, FN = false negatives).

**ISHLT Grade of rejection**

**0, 1 & 2            3**

<b>Single site pathologist assignment</b>	<b>0, 1 &amp; 2</b>	TN = 557	FN = 19
	<b>3</b>	FP = 17	TP = 14
<b>Breath test assignment</b>	<b>0, 1 &amp; 2</b>	TN = 600	FN = 17
	<b>3</b>	FP = 419	TP = 25

	<b>Site pathologist</b>	<b>Breath test</b>
Sensitivity	14/33 = 42.4%	25/42 = 59.5%
Specificity	557/574 = 97.0%	600/1019 = 58.8%
Positive predictive value	14/31 = 45.2%	25/444 = 5.6%
Negative predictive value	557/576 = 96.7%	600/617 = 97.2%

**Conclusions:** A breath test for markers of oxidative stress was more sensitive and less specific for Grade 3 heart transplant rejection than a biopsy reading by a single site pathologist, but the negative predictive values of the two tests were similar. A screening breath test may provide supportive information to help identify heart transplant recipients who are at low risk for grade 3 rejection.

**Supplies for the Heartsbreath test:** For each patient, sample collection for the Heartsbreath test requires:

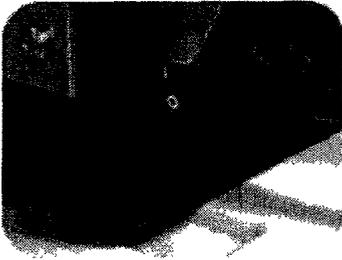
- Breath collection apparatus
- Disposable mouthpiece unit (comprising mouthpiece and T-connector with valves)
- Two sorbent traps with Swagelock seals

The Breath collection apparatus is supplied separately from the disposables (mouthpiece unit and sorbent traps).

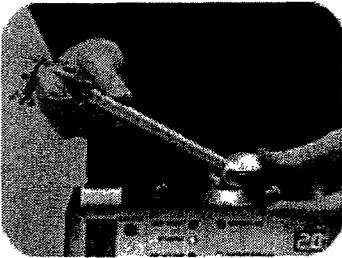
**Directions for use:**



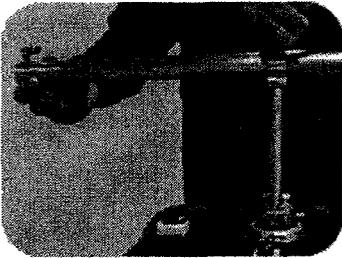
1 Connect power cord to the BCA and plug the unit into a power outlet.



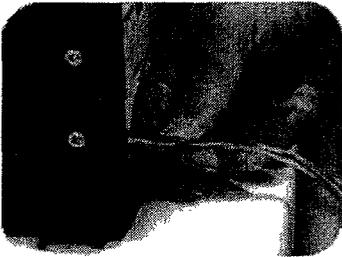
2 Switch on the unit; allow it to come to the "Ready" status.



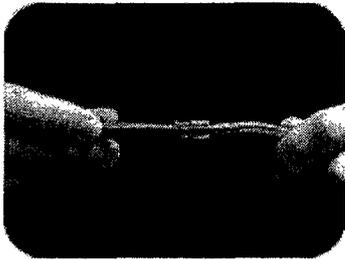
3 While the unit is warming up, loosen the reservoir adjustment knob and swing the reservoir support arm to a vertical position. Tighten the reservoir adjustment knob when the support arm is in the desired position.



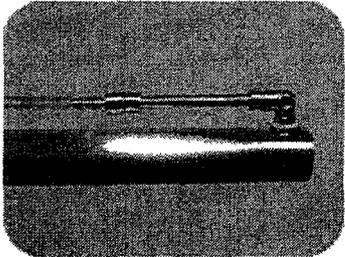
4 Loosen the butterfly nut to allow the reservoir to slide into the support arm's grip (portless end first). Tighten the butterfly nut until the grip is firm.



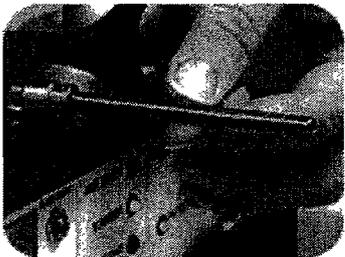
5 Attach the clear Tygon tube to the breath collection vacuum port if it is not already attached.



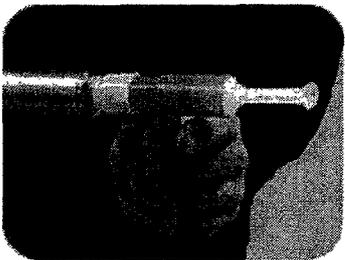
6 The steel adapter on the Tygon tube is then snapped onto the sorbent trap at the end with no lines etched into it.



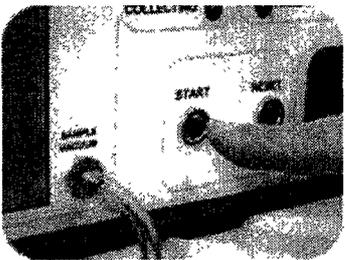
7 The sorbent trap is then snapped into the breath collection port by the end with the lines etched into it.



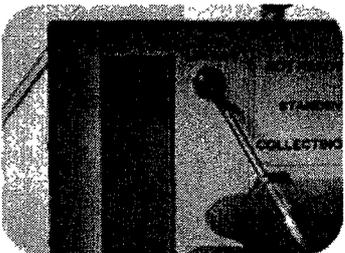
8 The sorbent trap for air collection is then snapped into the air collection port by the end with no lines etched into it.



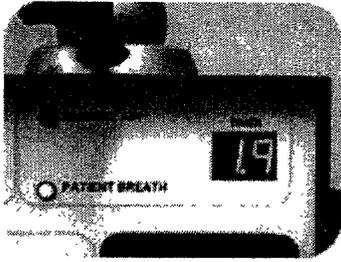
9 The mouthpiece is then connected into the reservoir at the end farthest from the breath collection port.



10 The volunteer must wear a nose clip and breathe into the mouthpiece at a normal rate. After the volunteer puffs into the mouthpiece a few times, press the "Start" button.



11 The flow meter must read 0.5 liters/minute. If it does not, adjust the rate using the flow rate adjustment screw. You will need to use a small flathead screwdriver.



- 12 The timer will count down from 2 min. and the "Patient Breath" light will be lit. After 2 min., the "Ambient Air" light will go on accompanied by a "beep" sound. At this point, the volunteer may leave.



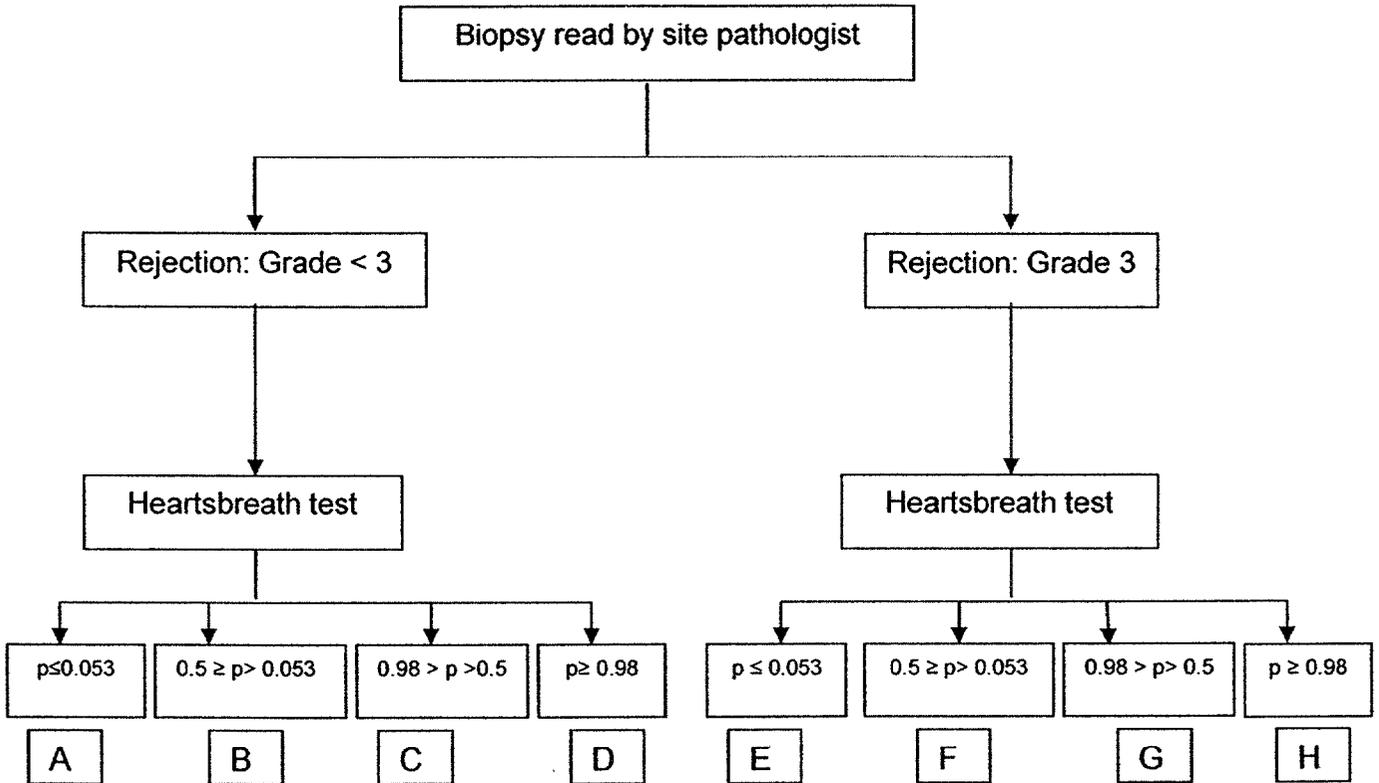
- 13 When the collection is finished, cap the sorbent traps firmly and ready them for shipping to the lab.

### Instructions for proper collection of breath samples:

- 1. Before the breath collection:** Ensure that the patient is relaxed and seated comfortably in the test area for at least ten minutes prior to the breath collection. Confirm that the patient has not eaten any food or smoked any tobacco products since the preceding midnight.
  - **Interfering substances:** Patients should not smoke or use tobacco products from midnight prior to the breath test. The breath test is not affected by ethanol (alcohol), toothpaste, deodorants, mouthwash or chewing gum.
  - **Fasting prior to the test:** The patient should fast from midnight prior to the breath test, though there is no restriction on consumption of water. The number of hours of fasting is not of critical importance. Patients are required to fast on the day of the breath test in order to ensure that their physiological state is similar to those of the subjects in the HARDBALL study, all of whom were similarly fasted. Fasting eliminates any potential confounding effect of food on breath markers of oxidative stress and grade 3 rejection.
- 2. Set up the BCA for the breath collection.** Follow the instructions described above in steps 1 through 9 under "Operating instructions for the breath collection apparatus (BCA)". Adjust the position of the breath reservoir and mouthpiece to ensure that the patient is in a comfortable position to put the mouthpiece in his or her mouth.
- 3. Collecting the breath sample:** Affix a nose clip to the patient's nose and then ask the patient to start breathing in and out through the mouthpiece. Make sure that the patient's lips are firmly sealed around the mouthpiece, and that no air is leaking in or out around the seal. When the patient is breathing easily in and out through the mouthpiece, follow the instructions described above in steps 10 through 13 under "Operating instructions for the breath collection apparatus (BCA)".

**Guidelines for interpretation of the Heartsbreath test when it agrees or disagrees with endomyocardial biopsy report:**

For the purpose of this scheme, Box A represents agreement in grade less than 3 rejection; Box H represents agreement in grade = 3 rejection. Boxes B through G represent varying probabilities of disparity between biopsy as read by pathologists and breath test results. For the purpose of interpretation of A through H, "p" represents the probability of grade 3 rejection. The discrepant biopsies should be evaluated by a second pathologist.



**Outcome A: Rejection: Grade < 3 on biopsy, Heartsbreath  $p \leq 0.053$**

Observation: Endomyocardial biopsy was negative for grade 3 rejection and Heartsbreath was negative for grade 3 rejection ( $p \leq 0.053$ )

Interpretation: Endomyocardial biopsy and Heartsbreath were concordant  
Patient does not have grade 3 rejection

**Outcome B: Rejection: Grade < 3 on biopsy, Heartsbreath  $0.5 \geq p > 0.053$**

Observation: Endomyocardial biopsy was negative for grade 3 rejection and Heartsbreath was intermediate for grade 3 rejection ( $0.5 \geq p > 0.053$ )

Interpretation: Endomyocardial biopsy and Heartsbreath were minimally discordant  
Patient probably does not have grade 3 rejection

**Outcome C: Rejection: Grade < 3 on biopsy, Heartsbreath  $0.98 > p > 0.5$**

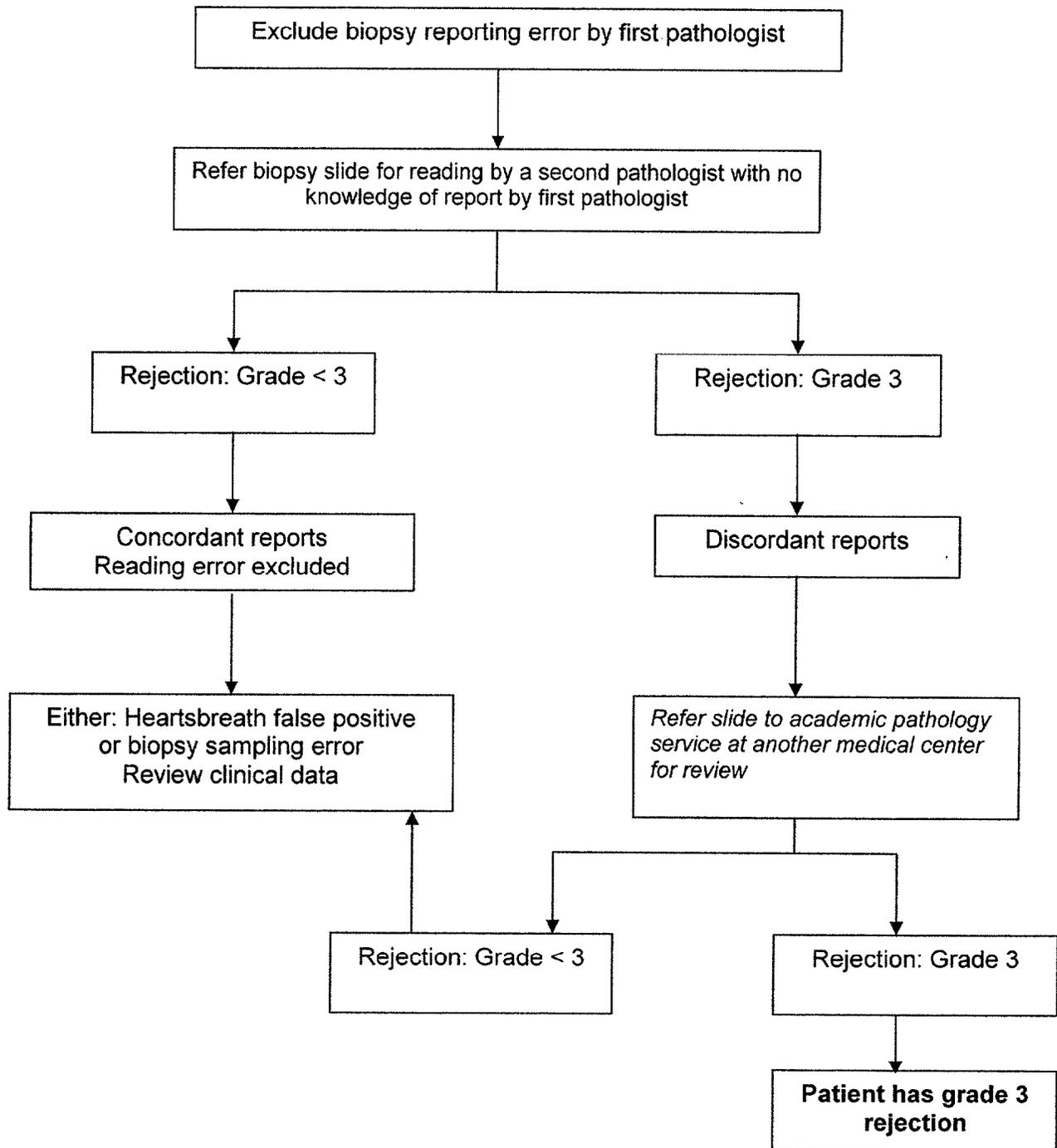
Observation: Endomyocardial biopsy was negative for grade 3 rejection and Heartsbreath was intermediate for grade 3 rejection ( $p > 0.5$ )

Interpretation: Endomyocardial biopsy and Heartsbreath were discordant

Possible causes:

- a. Endomyocardial biopsy was false negative  
due to - reading error by pathologist, or  
- sampling error in biopsy
- b. Intermediate Heartsbreath result was false positive

Recommended procedure



**Outcome D: Rejection: Grade < 3 on biopsy, Heartsbreath  $p \geq 0.98$**

Observation: Endomyocardial biopsy was negative for grade 3 rejection and Heartsbreath was positive for grade 3 rejection ( $p \geq 0.98$ )

Interpretation: Endomyocardial biopsy and Heartsbreath were discordant

Possible causes:

- a. Endomyocardial biopsy was false negative due to - reading error by pathologist, or  
- sampling error in biopsy
- b. Positive Heartsbreath result was false positive

Recommended procedure: Employ same decision tree as for Outcome C

**Outcome E: Rejection: Grade 3 on biopsy, Heartsbreath  $p \leq 0.053$**

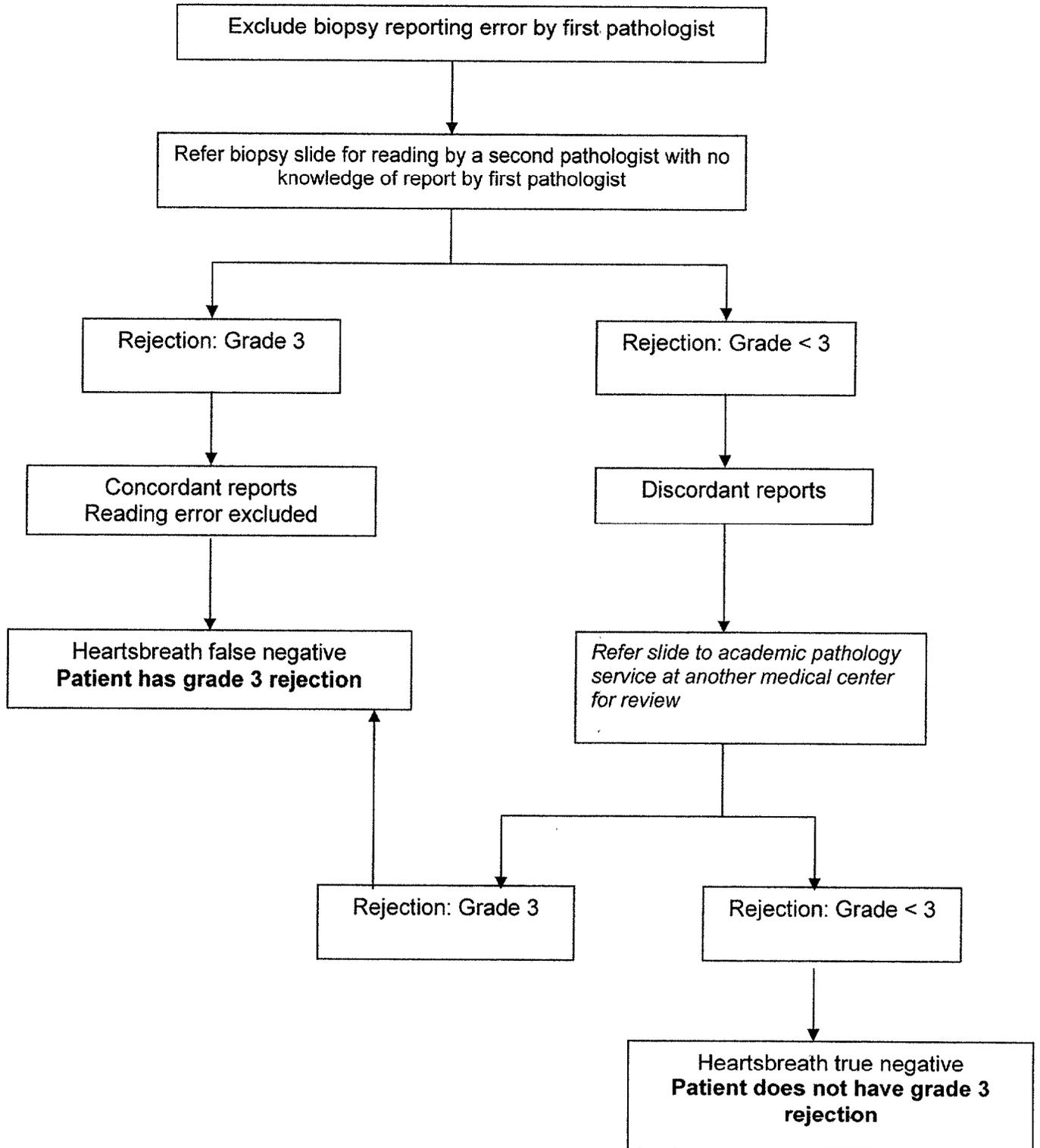
Observation: Endomyocardial biopsy was positive for grade 3 rejection and Heartsbreath was negative for grade 3 rejection ( $p \leq 0.053$ )

Interpretation: Endomyocardial biopsy and Heartsbreath were discordant

Possible causes:

- a. Endomyocardial biopsy was false positive due to reading error by pathologist
- b. Negative Heartsbreath result was false negative

Recommended procedure



**Outcome F: Rejection: Grade 3 on biopsy, Heartsbreath  $0.5 \geq p > 0.053$**

Observation: Endomyocardial biopsy was positive for grade 3 rejection and Heartsbreath was negative for grade 3 rejection ( $0.5 \geq p > 0.053$ )

Interpretation: Endomyocardial biopsy and Heartsbreath were discordant

Possible causes:

- a. Endomyocardial biopsy was false positive due to reading error by pathologist
- b. Negative Heartsbreath result was false negative

Recommended procedure: Employ same decision tree as for Outcome E

**Outcome G: Rejection: Grade 3 on biopsy, Heartsbreath ( $0.98 > p > 0.5$ )**

Observation: Endomyocardial biopsy was positive for grade 3 rejection and Heartsbreath was intermediate for grade 3 rejection ( $p > 0.5$ )

Interpretation: Endomyocardial biopsy and Heartsbreath were minimally discordant. Patient probably has grade 3 rejection

**Outcome H: Rejection: Grade 3 on biopsy, Heartsbreath  $p \geq 0.98$**

Observation: Endomyocardial biopsy was positive for grade 3 rejection and Heartsbreath was positive for grade 3 rejection ( $p \geq 0.98$ )

Interpretation: Endomyocardial biopsy and Heartsbreath were concordant. Patient has grade 3 rejection

**Risk/ probable benefit analysis:** The direct risk of performing the Heartsbreath test is minimal, because collecting breath is a non-invasive and intrinsically risk-free procedure. The only risk to the patient is cross infection, which can be greatly minimized by replacing the mouthpiece unit before every breath collection. Another risk may arise from the interpretation of the Heartsbreath test because a result may conflict with a biopsy report. This risk is minimized by recommending secondary biopsy review by a second pathologist prior to considering any change in treatment.

These risks must be balanced against the risks of *not* using the Heartsbreath test: a patient might get the wrong treatment because of a false positive or a false negative endomyocardial biopsy report. Biopsy reports can be skewed by two kinds of error. First, there may be a sampling error because rejection tends to be a patchy process, and a biopsy sample may show a lower intensity of rejection than the rest of the heart<sup>5</sup>. Second, the grading of rejection in a heart transplant biopsy is subjective and may therefore be influenced by observer bias. This contention is supported by studies which found high inter-observer variability in scoring the severity of heart transplant rejection<sup>6</sup>.

The Heartsbreath test is an objective test that is not skewed by observer bias. The major potential benefit of the Heartsbreath test is that it may reduce the risk of a patient getting the wrong treatment because of an erroneous biopsy report. These benefits are of two kinds: first, the Heartsbreath test may help identify patients with grade 3 rejection and a false negative biopsy report, which may help protect them from undertreatment of a life-threatening condition. Second, the Heartsbreath test may help identify patients with a false positive biopsy report who do not have grade 3 rejection, and may help protect them from the hazards of unnecessary treatment with steroids and other immunosuppressant medications.

## **Bibliography:**

1. Phillips M, Cataneo RN, Greenberg J, Gunawardena R, Naidu A, Rahbari-Oskoui F. Effect of age on the breath methylated alkane contour, a display of apparent new markers of oxidative stress. *J Lab Clin Med* 2000; 136:243-9.
2. Phillips M, Boehmer J, Cataneo R, et al. Heart Allograft Rejection: Detection with Breath Alkanes in Low Levels (the HARDBALL study). *J American College of Cardiology* 2002; 1:12-13.
3. Phillips M, Boehmer J, Cataneo R, et al. Heart Allograft Rejection: Detection with Breath Alkanes in Low Levels (the HARDBALL study). *The Journal of Heart and Lung Transplantation* (accepted for publication).
4. Billingham ME, Cary NR, Hammond ME, et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart Rejection Study Group. *The International Society for Heart Transplantation. J Heart Transplant* 1990; 9:587-93.
5. Nakhleh RE, Jones J, Goswitz JJ, Anderson EA, Titus J. Correlation of endomyocardial biopsy findings with autopsy findings in human cardiac allografts. *J Heart Lung Transplant* 1992; 11:479-85.
6. Winters GL. The challenge of endomyocardial biopsy interpretation in assessing cardiac allograft rejection. *Curr Opin Cardiol* 1997; 12:146-52.

## Patient Information about the Heartsbreath Test

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### *Intended use/ Indications for use*

The Heartsbreath test is indicated for use as an aid in the diagnosis of grade 3 heart transplant rejection in patients who have received heart transplants within the preceding year. The Heartsbreath test is intended to be used as an adjunct to, and not as a substitute for, endomyocardial biopsy. The use of the device is limited to patients who have had endomyocardial biopsy within the previous month.

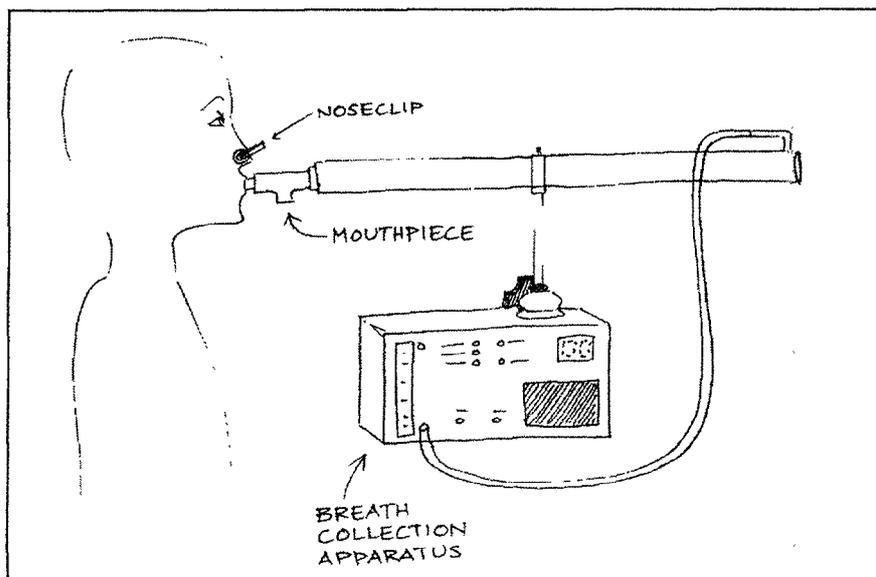
### *Heart transplant rejection*

After a heart transplant, the body sometimes tries to get rid of the new heart. That is called heart transplant rejection, and it can be very dangerous. You are probably taking drugs to prevent heart transplant rejection, and your doctor needs to know whether these drugs are working properly. That is why your doctor does a heart biopsy (also known as an endomyocardial biopsy) by taking a small piece of your heart and then looking at it under a microscope. Doctors grade the heart transplant rejection by how severe it looks under the microscope: grade 0 is no rejection at all, grade 1 is mild rejection, grade 2 is moderate rejection, and grade 3 is severe rejection. Grade 4 is very severe rejection, but it is unusual nowadays. Generally, doctors only need to change your drugs if the biopsy shows grade 3 heart transplant rejection.

The Heartsbreath test measures chemicals in your breath which tell if you have grade 3 heart transplant rejection or no rejection at all. It is not used in place of a biopsy – your doctor uses the Heartsbreath test and the biopsy together, to judge whether you have grade 3 rejection. Your doctor cannot use the Heartsbreath test if you have not had a recent biopsy or if you have had your new heart for more than a year.

### *How the Heartsbreath test is used*

The Heartsbreath instrument collects chemicals from your breath. This picture shows a person breathing into the Heartsbreath instrument (it is called a breath collection apparatus).



You will be asked to do the same thing as in the picture. You put on a nose clip so your breath passes through the mouthpiece of the Heartsbreath instrument. You breathe easily in and out through the mouthpiece for two minutes. The mouthpiece does not resist your breathing. You do not have to work hard – just breathe normally. Each patient uses a new mouthpiece. This helps you avoid exposure to germs from a previous patient.

After you have breathed into the Heartsbreath instrument, the lab tests the chemicals from your breath. The lab compares chemicals from your breath with chemicals from the breath of other transplant patients. Some of the other patients had grade 3 rejection, so the lab can tell your doctor whether the chemicals in your breath match those of other patients with grade 3 rejection. Your doctor compares your Heartsbreath results with your biopsy results to see whether the two tests agree with each other.

### ***When the Heartsbreath test should not be used (Contraindications)***

There are no known risks to breathing into the Heartsbreath instrument.

### ***Preparing for the day of the test***

- Do not eat after midnight before your test. If you do, the test results will not be useful. You may drink water, chew gum, and use toothpaste, mouthwash, and deodorant after midnight.
- Do not smoke or use other kinds of tobacco after midnight before your test. If you do, your test results will not be useful.
- Do not stop taking your prescribed medicines.

### ***Checklist for the day of the test***

- Have you smoked or used any tobacco products since midnight?
- Have you had anything to eat since midnight?
- If you have used tobacco products of any kind or consumed any food since midnight, you must inform the Heartsbreath technician.

### ***Important Information about the Heartsbreath test***

- The Heartsbreath test is not a substitute for endomyocardial biopsy – your doctor uses it only with a biopsy. Your doctor will use both tests together in combination, to help find out whether or not you are suffering from grade 3 heart transplant rejection.
- Breath collection is safe. There are no known risks to breathing into the Heartsbreath instrument. You will use a new mouthpiece, so there is no risk of germs from other patients.
- It is easy to breathe in and out of the Heartsbreath instrument, so the test is not uncomfortable.

### ***Risks of the Heartsbreath test***

- The Heartsbreath technician installs a clean mouthpiece for you. If the operator fails to do this, you may get germs from a previous patient.
- Your Heartsbreath test could be wrong and mislead your doctor if you may have different breath chemicals from other patients with the same amount of rejection.

### ***Benefits of the Heartsbreath test***

- The biopsy test can be wrong. If your biopsy test shows grade 3 transplant rejection, the Heartsbreath test can help you be sure it is right. Using the Heartsbreath test and your biopsy test together helps you get the correct treatment.

### ***Postoperative care with the Heartsbreath test***

Your doctor will get your Heartsbreath test results in about 2 days. Your result will be “positive”, “negative”, or “intermediate”. An intermediate result will have a probability number.

- A negative Heartsbreath test and a negative biopsy means you probably do not have grade 3 rejection.
- A positive Heartsbreath test and positive biopsy means you probably do have grade 3 rejection.
- Any other result means you may or may not have grade 3 rejection.

### ***Importance of the need to adhere to a care regimen***

- You and your physician are partners in your care. Grade 3 heart transplant rejection is a hazardous and potentially life-threatening condition, so it is essential for you to work together with your physician to reach the correct diagnosis and to adhere to the plan of treatment that is prescribed for you.