

# Erythromycin Breath Test

## *In Vivo Measurement of CYP3A4 Activity*

**Caution: Federal law prohibits dispensing without prescription.**

**Caution: Radioactive Material**

To be administered in compliance with the requirements of Federal regulations regarding radioactive drugs for research use (21 CFR361.1).



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ERMBT Package Insert Revision 6 Effective Date: 1/2/2003

## Intended Use

The erythromycin breath test (ERMBT™) is a rapid, quantitative measure of *in vivo* cytochrome P450 3A4 (CYP3A4) activity. The test is for professional use as a investigational tool for assessing CYP3A4 activity during pharmacological research. The user will conduct the test, collect the required breath samples and send the breath samples to the laboratories of Metabolic Solutions for analysis. A report containing the percentage of erythromycin metabolized per hour will be sent to the user.

There are two clinical indications for the ERMBT. The ERMBT is indicated for monitoring CYP3A4 before, during and after treatment of patients with pharmaceuticals, including new chemical entities, or other xenobiotic compounds which might alter CYP3A4 activity. The second indication for the ERMBT is for stratifying (phenotyping) a clinical population to determine relative interpatient CYP3A4 activity during pharmacokinetic, drug metabolism and pharmacodynamic studies.

## Summary and Explanation of the Test

Cytochrome P450s are a supergene family of microsomal enzymes that play a critical role in metabolism and clearance of many medications. Major P450s involved in drug metabolism are most abundant in the liver and are classified into four distinct gene families termed CYP1, CYP2, CYP3 and CYP4. Significant interindividual differences in the liver content and catalytic activities of many P450s (1) can explain differences in drug kinetics and/or susceptibility to adverse drug reactions between patients (2).

Cytochrome P450 3A4 (CYP3A4) is the most abundant cytochrome P450 within the liver accounting for approximately one-third of the total P450s present in adult liver (1). CYP3A4 is capable of metabolizing many categories of important drugs including:

- immunosuppressants
- calcium channel blockers
- cancer chemotherapeutic agents
- antihistaminics
- synthetic estrogens
- reverse transcriptase inhibitors
- sedatives
- HMG CoA reductase inhibitors

It is estimated that up to one-third of all orally administered pharmaceuticals currently in use, and up to one-third of new chemical entities (NCE) under development, are metabolized by CYP3A4.

There are two important implications for finding CYP3A4 substrates. First, CYP3A4 substrates are susceptible to certain drug interactions because CYP3A4 is induced or inhibited by many commonly used medications. For example, the immunosuppressant cyclosporin A and the antihistamine terfenadine are metabolized chiefly by CYP3A4 (3,4). Toxic reactions may develop (4,6) when patients receiving these drugs are co-administered ketoconazole or erythromycin, which inhibit CYP3A4 activity (5). Conversely, CYP3A4 is inducible by treatment with antiseizure drugs and the antibiotic rifampin (5). This explains why patients treated with these medications require increased dosing of cyclosporin A (6). In drug development, the discovery that a NCE is largely metabolized by CYP3A4 suggests the potential for similar drug interactions.

Second, large interpatient differences exist in the content and catalytic activity of CYP3A4. Examination of liver biopsies reveal 30-fold interpatient differences in CYP3A4 expression (1). These differences exist in the absence of medications known to influence CYP3A4 enzyme regulation and may largely reflect genetic or dietary factors (2). This interpatient CYP3A4

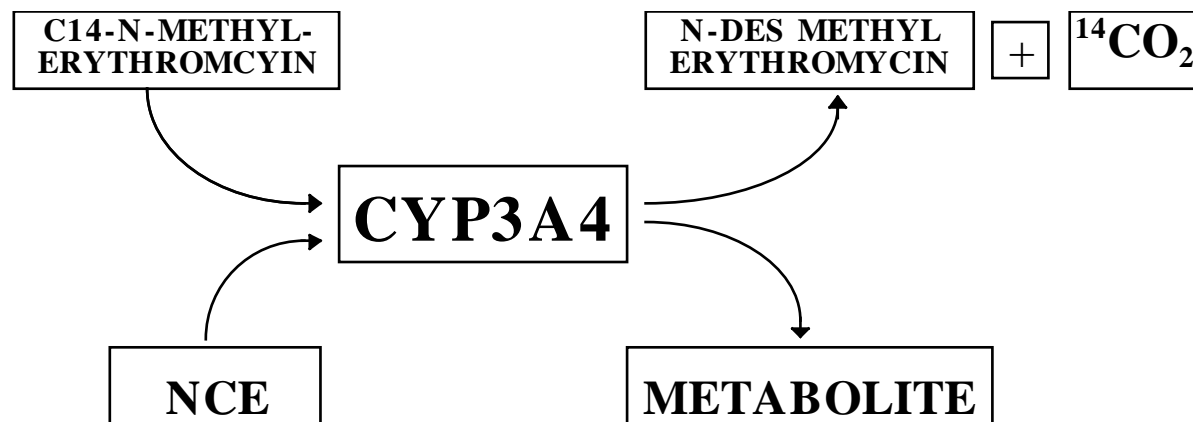
variability has been shown to account for differences in the kinetics of certain drugs (7). Since liver CYP3A4 activity appears to be rate limiting in the elimination of many drugs, knowledge that a NCE is metabolized by CYP3A4 may therefore imply that significant interpatient differences in elimination kinetics will be observed.

The most promising non-invasive means of measuring liver CYP3A4 activity in patients is to use model substrates of CYP3A4 as *in vivo* probes. The erythromycin breath test (ERMBT) has been validated as a model drug probe for measuring CYP3A4 activity (7). Erythromycin is N-demethylated by CYP3A4 and the demethylated carbon ultimately appears as breath carbon dioxide. If the N-methyl carbon of erythromycin is labeled with carbon-14, the activity of CYP3A4 can be determined from changes in the rate of production of  $^{14}\text{CO}_2$  in the breath.

### Principle of the Test

The ERMBT™ uses a 3  $\mu\text{Ci}$  [C-14 N-methyl] erythromycin dose as a substrate for CYP3A4 enzyme. The substrate is injected intravenously into a hand or arm vein for rapid distribution in the body. Predominately liver CYP3A4 metabolizes the erythromycin via N-demethylation of the labeled methyl group to produce formaldehyde. The formaldehyde is converted rapidly to carbon dioxide by ubiquitous enzymes in the body. The labeled carbon dioxide appears in the breath. A single breath collection at 20 minutes after injection of the test dose of erythromycin estimates the percentage of administered radiolabel exhaled. The percent of carbon-14 appearance in the breath at 20 minutes correlates with the percent erythromycin metabolized per hour. A correlation equation derives a relative percent erythromycin metabolized which can be used to compare interpatient or inpatient differences after various experimental treatments.

The principle of the test and its application in clinical trials is illustrated below:



If CYP3A4 activity is rate limiting *in vivo*, the results usually correlate with the fractional clearance of a NCE through a CYP3A4 catalyzed pathway.

## Materials

### Material provided:

- 3  $\mu\text{Ci}$  [ $^{14}\text{C}$  N-methyl] erythromycin in 0.5 ml ethyl alcohol 100%, USP (provided in separate box). Store at  $-20\text{ }^{\circ}\text{C}$  until needed.
- 5% Dextrose Injection, USP
- 2 - balloons for breath collections
- 2 - straws for breath collections
- 2 - Alcohol swab
- Blood collection set
- Syringe, 5 cc with 21 gauge needle
- Syringe, 3 cc with Luer slip tip
- Return breath sample shipping box
- Patient identification sheet

### Material required but not provided:

- Timer
- Bandages
- Shipping box to return multiple breath test samples for analysis

## Precautions

1. For investigational use only.
2. Users of the test are required to receive approval from Investigational Review Boards (IRBs) to administer the ERMBT™ in clinical studies. In addition, users must either amend an Investigational New Drug (IND) application with the U. S. Food and Drug Administration (FDA) or receive approval from a Radioactive Drug Research Committee (as noted in 21 CFR 361.1). Users may contact Metabolic Solutions for access to the contents of a Device Master File submitted to the FDA.
3. **Caution:** Observe Federal and State guidelines for handling radioactive material contained in this kit. Users must have a valid license to receive and use carbon-14 radioactive material and a license to administer radiomaterials to humans.
4. All radioactive contaminated material must be disposed by the user according to applicable Federal and State guidelines.
5. DO NOT use any sterile component of the kit that appears contaminated, tampered with or not sealed.
6. DO NOT use any reagents from a container that appears to have leaked.

7. DO NOT use erythromycin dose or dextrose injection solution beyond expiration date.
8. Use breath collecting device only once.

### **Contraindications**

The ERMBT is contraindicated for:

- persons less than 18 years of age
- pregnant females
- nursing mothers

The ERMBT should not be administered to one person more than 10 times per year.

### **Storage and Stability**

Store the Erythromycin dose at -20°C. Avoid prolonged exposure (> 3 hours) to higher temperatures prior to administering dose. The erythromycin dose is stable for 2 years from the date of manufacture. All vials contain the expiration date.

Store the other kit components at room temperature (15° to 30°C, 59° to 83°F). The dextrose injection solution is stable for 1 year from the date of manufacture. The expiration date of the dextrose injection solution is printed on the container.

### **Test Procedures**

#### **1. Insertion of Intravenous Butterfly**

A small butterfly needle and tubing assembly will be used to administer the dose. The butterfly needle can either be inserted into a hand or arm vein. Insert the butterfly assembly as follows:

1. Open the butterfly blood collection set. Remove the luer adapter from end and discard.
2. Stretch the tubing by holding butterfly needle in one hand and pull other end to prevent kinks in the line.
3. Wipe area of needle insertion (hand or arm vein) with alcohol swab.
4. Insert needle into vein until blood flows into tubing. Insert the 3 ml syringe (without needle) on the luer end of the tubing. Check for patentability of line by withdrawing a small amount of blood into the line.

#### **2. Preparation of Dose**

Prepare the erythromycin dose as follows:

1. Wear disposable gloves when preparing dose.
2. Wipe the port of the 5% Dextrose Injection Solution with an alcohol swab.
3. Use the 5 ml syringe with needle (provided with kit) to draw 4.5 ml of Dextrose Injection solution.
4. Remove cap on the [<sup>14</sup>C N-methyl] erythromycin dose vial.

5. Wipe top of erythromycin vial with alcohol swab.
6. Inject 4.5 ml Dextrose solution into erythromycin vial, periodically allowing air to escape back into the syringe to prevent pressure build-up.
7. Invert vial gently 2-3 times to mix contents.
8. Draw 5 ml of air into syringe. Inject into the erythromycin vial.
9. Holding the vial upside down, using a series of air for solution exchanges, draw as close to 5 ml as possible of the erythromycin/dextrose solution with the same syringe from step 8. Be consistent in removing as much as possible from the vial for each test. Allow the needle tip to just pierce the septum to obtain the last drop of solution.
10. Holding the syringe upside down, draw solution from needle. Fill the syringe with about 0.5 cc air. Use the solution within 3 hours.

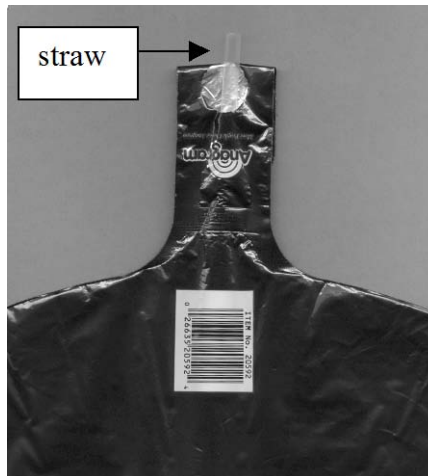
### 3. Collecting Breath Samples

Breath samples are collected using the following procedure:

- a) Unwrap a straw and insert into the balloon at the opening. The straw fits between the blue and silver foil lining.



- b) Insert the straw completely into the balloon. A little pressure may be required to insert the straw into the valve. The straw should stick out not more than 1 inch from the end of the balloon.



- c) Inflate the balloon by exhaling normally into the straw.
- d) Completely fill the balloon up. This takes 3 to 6 exhalations. When the balloon is filled to capacity, a clinical assistant should quickly pull the balloon completely away from the straw but keep the straw in the subject's mouth. The air will not escape from the balloon. It is all right if the balloon is not filled to complete capacity. Do not re-insert the straw in the balloon.

#### 4. Breath Test Protocol

The breath test is conducted using the following procedure:

1. Insert the syringe containing the dose into the luer adapter. Inject the dose of  $^{14}\text{C}$  erythromycin into the vein over a period of approximately 60 seconds, along with enough of the 0.5 cc of air to flush the tubing (to ensure complete dose administration).
2. Start a 20 minute countdown after all the dose is injected into the vein.
3. Remove the IV butterfly.
4. Complete the label on each balloon. Fill out the patient ID form after test.
5. Collect two breath samples at 20 minutes. Use a new straw for each collection. Instruct the patient to exhale normally into the breath collection device. Fill the balloon with 3-6 breaths until the bag is full. The patient will not be able to fill the balloon further when full.
6. Ship **one** balloon back to Metabolic Solutions for analysis. Keep the other balloon as a duplicate in case a problem occurs. **Include the dose vial in the kit box.** The amount of radioactivity left in the vial will be determined by Metabolic Solutions.

7. Ship the samples to: Metabolic Solutions, Inc.  
460 Amherst Street  
Nashua, NH 03063
8. Use the kit box to protect filled balloons and dose vial when shipping samples to the laboratory. It is acceptable to place several kit boxes into a larger shipping box to reduce shipping costs. Include a patient ID form with each breath test sent.
9. The test is complete. Dispose of all supplies which are contaminated with radioactivity in proper radioactive storage containers.

### References

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6. Watkins PB. (1990) The role of cytochrome P-450 in cyclosporine metabolism. *J. Am. Acad. Dermatol.* 23:1301-1311.
7. Watkins PB. (1994) Noninvasive tests of CYP3A enzymes. *Pharmacogenetics* 4:171-184.