**Sputum analysis of healthy volunteers**

## Clinical trial

The goal of the study was to determine if sputum samples from healthy volunteers contain red fluorescent cells (RFC) after CyPath® labeling. This minimal risk study was registered with Clinicaltrials.gov (http://clinicaltrials.gov/ct2/show/NCT02388074) and conducted according to ethical principles of the Declaration of Helsinki (v 1996) and Good Practice guidelines. The Quorum Institutional Review Board (Seattle, WA) reviewed and approved the protocol.

The study was performed at Radiology Associates of Albuquerque (RAA), New Mexico.

## Subjects (Figure 1)

Male and female participants were recruited who had never smoked and were free of known lung diseases. Participants were excluded from the study when they met any of the following criteria:

- Severe obstructive lung disease
- Angina with minimal exertion
- Pregnancy
- Have or have had cancer
- Worked in the mining industry

Twenty-seven volunteers were interviewed; two refused and 25 were eligible and signed consent. Two sputum samples were lost in transit, and one sputum sample was not returned. Twenty-two participants were evaluated by a cytotechnologist. Of these 22, three participants' PAP-stained slides were unreadable and 14 participants' samples were confirmed by PAP as inadequate. Only five slides were confirmed by PAP as adequate deep lung sputum samples, and these five samples were evaluated for the presence of RFCs.

Of the five participants, two were female (46 and 60 years of age) and three were male (40, 45 and 60 years of age).

## Study design

### *Sample collection*

Subjects did not undergo low-dose computed tomography (LDCT) or any medical consultations related to this trial. Subjects were provided with an Acapella® device and a collection cup and received instructions from study staff on how to use the device in accordance with manufacturer's instructions and how to expel the sputum sample into a sterile collection cup. Individuals were instructed to repeat this procedure at home for three consecutive mornings and to store the specimen cup in a cool, dark place or in a refrigerator. As expected, sputum collection was difficult for healthy individuals; some generated no sputum the first two days but were able to produce sputum on days three through five. These samples were accepted. Sputum samples were collected at the investigative site (RAA) and at participants' homes within one to

three days after collection was complete. Samples were given an identification number to blind participants' identities and shipped the same day to the laboratory for further processing the next day.

***Sample processing, PAP staining, CyPath labeling***

The same procedures were followed and the same equipment was used as described in the Materials and Methods section for the clinical trial NCT00894127.

**CyPath® readings**

Twelve slides of each subject were carefully scanned for the presence of RFCs, using the equipment as described for the participants in clinical trial NCT00894127.

Similar to the procedure used on the high-risk and cancer cohorts (NCT00894127), one slide from each subject was selected at random, and the average fluorescence intensity (FI) and cell size (area) were determined for 50 cells.

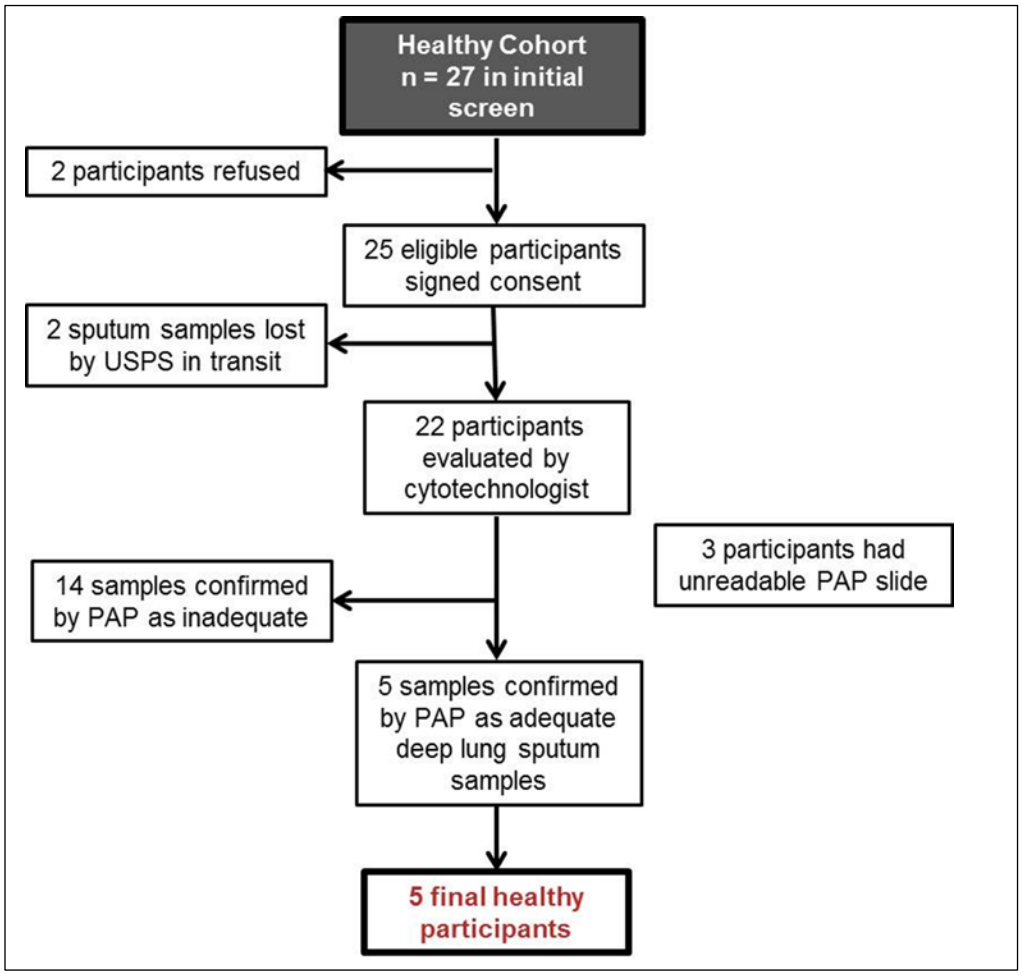


**Figure 1. Subject enrollment and Participation**