*Early*CDT®–Lung

IVD CE

Instructions for Use

Date of issue: 2018/05/16

Oncimmune Limited
Clinical Sciences Building
City Hospital
Hucknall Road
Nottingham,
NG5 1PB
United Kingdom

Phone: +44 0115 823 1869
earlycdt@oncimmune.co.uk
www.oncimmune.co.uk

Contents

Intended Use

The EarlyCDT-Lung test is intended to be used as an immunoassay for the *in vitro* detection of a panel of seven lung cancer autoantibodies in humans to aid in the early detection of lung cancer in high risk patients and risk stratification of patients with indeterminate pulmonary nodules identified by CT.

The EarlyCDT-Lung test is designed for professional use and analytical results should be interpreted by medical professionals in combination with all other available clinical information.

Introduction and Background to the Test

Lung cancer is a leading cause of all cancer related deaths worldwide affecting millions of patients annually. Lung cancer is responsible for 13% of cancer cases and 19% of all cancer deaths in both genders worldwide.[1] Tobacco smoking is the predominant cause of lung cancer, however the following are also risk factors:

- Age
- Gender
- Smoking history
- Emphysema/COPD
- Family history of lung cancer in first degree relatives
- Environmental exposure including dust, asbestos and ionising radiation including radon gas

Data from the National Lung Screening Trial[2] performed in the USA has shown that earlier detection through annual screening with low dose computed tomography (LDCT) results in a 20% improvement in lung cancer mortality. Hence earlier detection saves lives. However LDCT screening has disadvantages in that 96.4% of positive LDCTs are benign findings (false positives).[2] It also delivers a potentially harmful dose of radiation.

Tumour cells express proteins in altered or up-regulated forms compared with their normal (non-malignant) counterparts. These are known as tumour-associated antigens (TAAs) and some are shed into the circulation of the cancer patient. A number of researchers have proven that the cancer patient's immune system often recognises the altered state (e.g. mutation, over expression or aberrant glycosylation) of TAAs as non-self and mounts an antibody response against them. Thus this antibody response acts as an early amplified *in vivo* signal for the presence of TAA (and hence tumours) in the body. Such antibodies are known as autoantibodies (AAb) since they react with the host's own altered proteins and it is these that the EarlyCDT-Lung test is designed to measure. Hence the results of the EarlyCDT-Lung test can be used as an aid for early identification of the presence of lung cancer. In validation studies, the EarlyCDT-Lung test has demonstrated high specificity (>90%)[3, 4] and is non-invasive requiring only the drawing of a blood specimen.

Recommended Patient Groups

The EarlyCDT-Lung test is recommended for use in humans who are at high risk of lung cancer due to a combination of age, gender, smoking history and other risk factors such as environmental exposures (dust, asbestos, radioactive substances), those with a history of emphysema/COPD, or family history of lung cancer in a first degree relative.

Oncimmune's current recommendations are:

- Patients that are ≥50 years of age with at least a 20 pack year smoking history (equivalent to smoking one pack of cigarettes per day for 20 years)
- Patients that are 40-49 years of age with at least a 20 pack year history plus at least one additional risk factor (see introductory section).

The EarlyCDT-Lung test can also be used in conjunction with diagnostic imaging techniques to further assess the risk of lung cancer being present where indeterminate lung nodules have been detected but have not been diagnosed as malignant.

Limitations of Use

Patients with a previous history of cancer of any type should not take the EarlyCDT-Lung test. The exception to this recommendation is for patients with a history of basal cell carcinoma (BCC). A study was conducted, and the data suggested that BCC does not impact the EarlyCDT-Lung test result (data on file with Oncimmune).

The EarlyCDT-Lung test should not be used in patients known to have diseases that result in an elevated level of serum total protein, for example myeloma, amyloidosis, monoclonal gammopathy of undetermined significance (MGUS).

Test Principle

Patients with lung cancer can mount a humoral response to their disease[5-8] and autoantibodies have been described up to 4 years before clinical diagnosis in some cases.[9-11] The Oncimmune EarlyCDT-Lung test is for the *in vitro* detection of autoantibodies to a panel of seven lung cancer antigens (CAGE, GBU4-5, HuD, MAGE A4, NY-ESO-1, p53 and SOX2) that are present in the earliest stages of lung cancer. It is performed as an indirect Enzyme-Linked Immunosorbent Assay (ELISA). The reagents provided are used together for the measurement of the panel of the seven autoantibodies described above in up to 10 patient specimens. Plates are pre-coated with tumour associated antigens and a control protein (VOL control) at two dilutions: 50nM and 160nM, see figure 1. Diluted patient specimen is loaded into wells of the coated plate and incubated. Up to five patient specimens can be run on each plate supplied. Following a series of reagent addition, incubation and washing steps, the autoantibodies are finally detected by the addition of a colourimetric reagent, and the resulting signal is measured using a spectrophotometric plate reader.

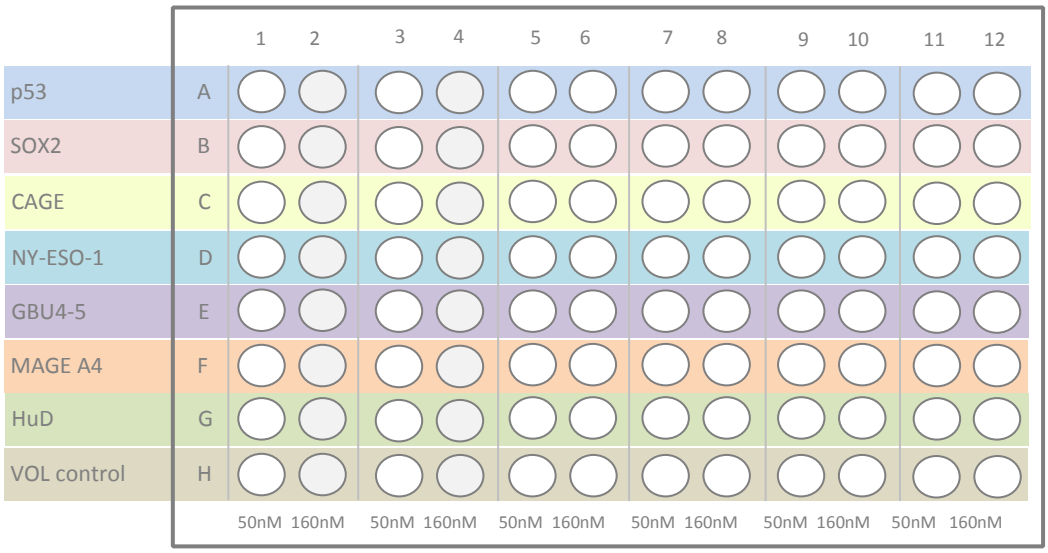

Figure 1: EarlyCDT-Lung microtitre plate layout. One entire row is coated with the same TAA, alternating between 50nM and 160nM protein in each well. The wells of row H are coated with a control protein to allow for correction for non-specific binding.

Reagents and Materials Provided

- Each EarlyCDT-Lung Test Kit contains the reagents listed in table 1, which are sufficient for a maximum of 10 or 8 tests depending on usage:
    - If both plates within a kit are being run at the same time, then up to 10 patient specimens can be run in total:
        - 5 patient specimens on plate 1 alongside Control A and 5 patient specimens on plate 2 alongside control B
    - If plates within the kit are being run on different occasions, then up to 8 patient specimens can be run in total:
        - 4 patient specimens on plate 1, alongside Control A and Control B
        - 4 patient specimens on plate 2, alongside Control A and Control B
    - Since partly used microtitre plates must be disposed of the most efficient use of this kit is achieved by running 4 or 10 patient specimens at the same time.
- The expiry date of the kit is stated on the label outside the box.
- DO NOT use the kit beyond the expiry date.
- DO NOT use the EarlyCDT-Lung Test Kit if the outer seal is broken upon receipt. Please contact Oncimmune if the outer seal is broken upon receipt.
- DO NOT use any opened or unopened reagents beyond their expiry date.
- DO NOT mix reagents from different kit lots.
- Store the kit at +2-8°C. DO NOT freeze.
- Opened reagents are stable according to table 1, provided they are not contaminated, are stored in resealed original containers and handled as described. Ensure all opened reagents are returned to +2-8°C immediately after use.

Table 1. EarlyCDT-Lung Test Kit components			
Part number	**Component**	**Quantity**	**Storage and stability after first use**
ECDTL2-SD	Specimen diluent	1x 27.5mL	+2-8°C for 4 weeks
ECDTL2-SA	Secondary antibody	1x 0.25mL	+2-8°C for 4 weeks (refers to undiluted stock)
	A polyclonal rabbit anti-human IgG antibody conjugated with horseradish peroxidase		
ECDTL2-AD	Antibody diluent	1x 27.5mL	+2-8°C for 4 weeks
ECDTL2-WB	Wash buffer (20x)	1x 55mL	+2-8°C for 4 weeks (refers to undiluted stock)
ECDTL2-MTP	96-well microtitre plates	x2 plates	Discard after first use
ECDTL2-CA	Control A	2x 1.8mL	+2-8°C for 4 weeks
	Human material containing autoantibodies formulated to be reactive to p53, SOX2, CAGE and NY-ESO-1		
ECDTL2-CB	Control B	2x 1.8mL	+2-8°C for 4 weeks
	Human material containing autoantibodies formulated to be reactive to MAGE A4, GBU4-5 and HuD		
ECDTL2-SEAL	Sealing strips	x4 strips	+2-8°C or room temperature until expiry date stated on the kit box
ECDTL2-SUB	Substrate	1x 27.5mL	+2-8°C for 4 weeks Keep out of direct sunlight
	Substrate for horseradish peroxidase		
ECDTL2-SS	Stop solution	1x 27.5mL	+2-8°C for 4 weeks
	Sodium fluoride solution at 1g/L		
ECDTL2-USB	USB device	x1	+2-8°C or room temperature
	Contains: Instructions for Use (ECDTL2-IFU); Lot Specific Insert (ECDT2-LSI); Material Safety Data sheet; and EarlyCDT-Lung Test result calculation software		

Equipment, Consumables and Materials Required But Not Provided

Table 2. Equipment, consumables and materials required but not provided	
Item	**Description**
1. Graduated measuring cylinder	50mL & 1000mL: For measuring and preparing wash buffer.
2. Distilled water	For preparation of wash buffer.
3. 50mL tube with cap	For use during secondary antibody dilution.
4. Pipettes	20µL: For patient specimen preparation. 100µL 8-channel pipette: For dispensing secondary antibody, substrate and stop solution into the wells in each column of the plate. 100-1000µL: For secondary antibody preparation and patient specimen dispensing. 1-5mL: For patient specimen and secondary antibody dilution. *All pipettes used must be calibrated so that accuracy of pipetting can be assured.
5. Pipette tips	Suitable for accurate dispensing with the above mentioned pipettes.
6. Reagent troughs	3 clean troughs required, up to 25mL capacity: For holding secondary antibody, substrate or TMB solution whilst pipetting with a multi-channel pipette.
7. Microtitre plate shaker	Capable of shaking at 400 revolutions per minute (RPM).
8. Microtitre plate washer or wash bottle	A dispensing wash bottle capable of holding 0.5L of solution. Or, an automatic plate washer capable of performing 4 consecutive wash cycles with a fill volume of 300µL/well. **Note:** Any automated plate washer used should be validated by the user.
9. Timer	For ensuring incubation steps are performed to the time specified in the assay protocol.
10. Absorbent paper	To remove any residual liquid from the wells following washing.
11. Microtitre plate reader	With a reading wavelength of 650nm.
12. PC with USB connection and Microsoft Excel 2007 or newer	For test result calculation using the USB device supplied with the kit.

Stability and Storage

The EarlyCDT-Lung Test Kit is stable until the expiry date stated on the box label when reagents are stored as recommended.

Unopened kit reagents should be stored as instructed on the individual reagent packaging and are stable as supplied until the kit expiration date. Once opened, each reagent is stable for the duration stated in table 1.

Indications of instability

The substrate should be colourless. A blue colour indicates that the reagent may have been contaminated and should be discarded.

Safety Precautions

For *in vitro* diagnostic use:

- Blood products are potentially infectious and should be handled, stored, and disposed of according to local biohazard regulations.
- Control reagents supplied with this kit are of human origin. They have been tested and found to be non-reactive for HIV-1/2 Antibody, HCV Antibody and Hepatitis B Surface Antigen (HBsAg). Since no method can completely rule out the presence of blood borne pathogens, the handling and disposal of the control reagents from this kit should be performed as if they were potentially infectious.
- The material safety data sheet (MSDS) provides detailed information relating to the correct disposal, handling and hazards that are associated with the EarlyCDT-Lung Test Kit. The MSDS is provided on the USB device supplied with the kit and is also available from Oncimmune (phone: +44 (0)115 823 1869 or email: earlycdt@oncimmune.co.uk).

Specimen Collection Handling and Storage

The EarlyCDT-Lung Test Kit is intended for use with human serum or plasma, collected in one of the following tubes:

- Serum: either clot activator or gel tubes.
- Plasma: collected using EDTA tubes.
- **Note:** Plasma collected in heparin and citrate tubes **should not be used**.

Collect blood by venepuncture and process according to the collection tube manufacturer's instructions to separate serum or plasma from clotted and cellular material. Whole blood specimens should be stored at room temperature (+18-25°C) and be processed within 4 days. If specimens of serum or plasma are not to be assayed immediately they can be stored at +2-8°C for 14 days or at -25°C to -15°C for up to 2 months. Specimens must not undergo more than 5 freeze thaw cycles. Bring frozen specimens to room temperature and mix thoroughly by gentle inversion before analysis.

Assay Protocol

Assay notes and precautions to read BEFORE starting an EarlyCDT-Lung Test Kit assay


Ensure that the assay is performed at +18-22°C.


Each timed incubation step must be performed exactly for the duration stated. As soon as one plate is filled a timer must be started.


All equipment used must be adequately maintained, calibrated and validated to ensure correct functioning.


Ensure reagents do not get mixed together by using clean troughs for each reagent.


Do not use the kit if the outer seal is broken upon receipt.


Do not use any kit reagents if they are damaged, appear to have leaked or are open.


Do not store reagents in packaging other than those in which they were received.


The EarlyCDT-Lung Test Kit has been developed to be performed by a competent laboratory technician.* Ensure a thorough understanding of the protocol is gained before starting an assay.


Dispose of solutions, especially those that contain biological material, according to local disposal regulations.


Reliability of assay results cannot be guaranteed if there are any deviations from the protocol provided in this document.

Please contact Oncimmune customer services by phone +44 (0)115 823 1869 or email earlycdt@oncimmune.co.uk for support, if the outer seal is broken upon receipt or the reagents are damaged or have leaked.

*A competent laboratory technician is defined as an individual with experience of running ELISAs and is trained and competent in carrying out general laboratory procedures, such as buffer preparation, pipetting and able to meticulously follow instructions.

Step 1: Reagent and Specimen preparation

⚠ Read Important Notes below associated with this step.

a) Record the LOT number stated on the kit box label and your specimen ID numbers:

Kit LOT number: _____ (this will be needed when using the result calculation software)

Plate 1	Specimen ID	Plate 2	Specimen ID
Specimen 1		Specimen 6	
Specimen 2		Specimen 7	
Specimen 3		Specimen 8	
Specimen 4		Specimen 9	
Specimen 5		Specimen 10	

b) Remove all components from the kit box and leave to equilibrate to +18-22°C for **at least two hours before use**.

c) Depending on the number of plates being used in the assay, prepare the assay reagents and patient specimens as follows:

Preparation	1 plate assay (Max 4 samples)	2 plate assay (Max 10 samples)
Wash buffer	Mix 25mL of Wash buffer (ECDTL2-WB) with 475mL of deionised water	Mix 50mL of Wash buffer (ECDTL2-WB) with 950mL of deionised water
Secondary antibody	Mix 0.12mL of concentrated antibody (ECDTL2-SA) with 12mL of antibody diluent (ECDTL2-AD)	Mix 0.24mL of concentrated antibody (ECDTL2-SA) with 24mL of antibody diluent (ECDTL2-AD)
Patient specimen	Mix 20µL of patient specimen with 2.2mL of specimen diluent (ECDTL2-SD)	

d) Remove either one or both microtitre plates from their foil pouches (Part Number: ECDTL2-MTP), depending on how many patient specimens you intend to run in your assay.

⚠ **Step 1 - Important Notes:**

- 1 of 5: Due to the high concentration of salt in the wash buffer supplied you may observe crystallisation. If this happens, simply warm prior to use to redissolve the salts by either placing the bottle directly into a water bath or holding the sealed bottle under warm running water until crystallisation is no longer visible.

- 2 of 5: It is recommended to centrifuge the vial of concentrated secondary antibody conjugate briefly (5-10 seconds) using a microfuge, or to tap the vial on the benchtop to dislodge any contents that may have become trapped in the lid of the vial during transport.
- 3 of 5: Invert patient specimens several times to mix prior to use.
- 4 of 5: Ensure a clean tip for each patient specimen is used.
- 5 of 5: Store reagents and specimens at room temperature on day of use, otherwise store at +2-8°C.

Step 2: Control and patient specimen incubation

a) Ensure that plates are orientated so that A1 well is at the top, left hand corner.
b) Depending on the number of plates being used in your assay, add the kit control and patient specimen to plate 1 and/or 2, as follows:

1 plate Assay

i. Following Plate 1 layout below, add 100µL of Control A (RED cap, Part Number: ECDTL2-CA) into every well of column 1 and 2.
ii. Add 100µL of Control B (BLUE cap, Part Number: ECDTL2-CB) into every well of column 3 and 4.
iii. Add 100µL of diluted patient specimen 1 into every well of column 5 and 6. REPEAT for patient specimens 2, 3 and 4 as shown for Plate 1.
iv. As soon as the plate has been filled, start a timer for 90min. Go to step c).

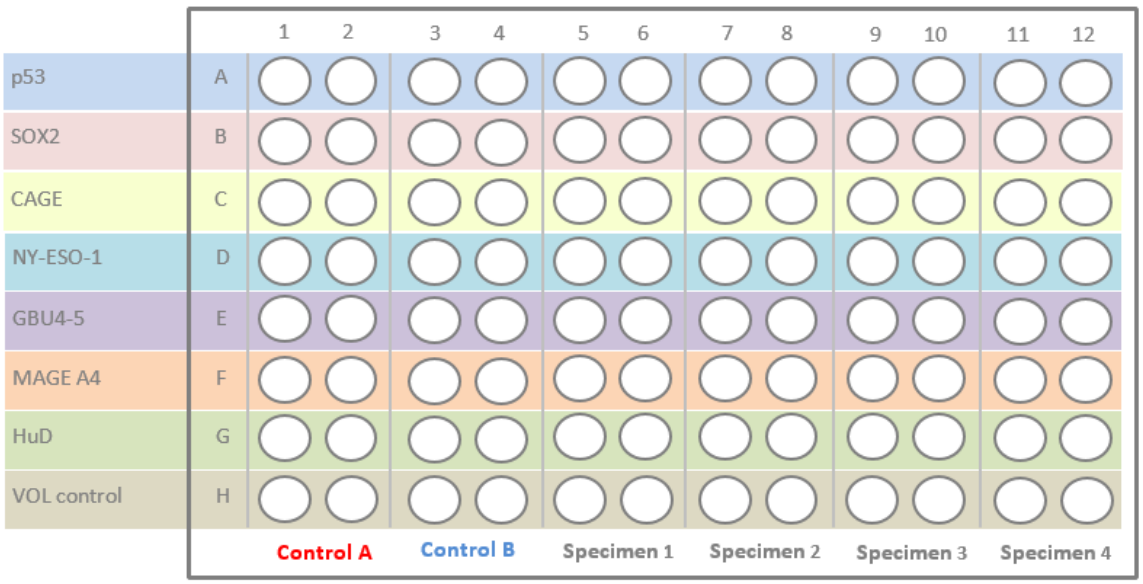

Plate layout when one microtitre plate is used in an assay.

2 plate Assay

i. Following Plate 1 layout below, add 100µL of Control A (RED cap, Part Number: ECDTL2-CA) into every well of column 1 and 2.
ii. Add 100µL of diluted patient specimen 1 into every well of column 3 and 4. REPEAT for patient specimens 2, 3, 4 and 5, as shown for Plate 1.
iii. As soon as Plate 1 has been filled, start a timer for 90min.
Following Plate 2 layout below, add 100µL of Control B (BLUE cap, Part Number: ECDTL2-CB) into every well of column 1 and 2.
iv. Add 100µL of diluted patient specimen 6 into every well of column 3 and 4. REPEAT for patient specimens 7, 8, 9 and 10 as shown for Plate 2.
v. As soon as Plate 2 has been filled, start another timer for 90min. Go to step c).

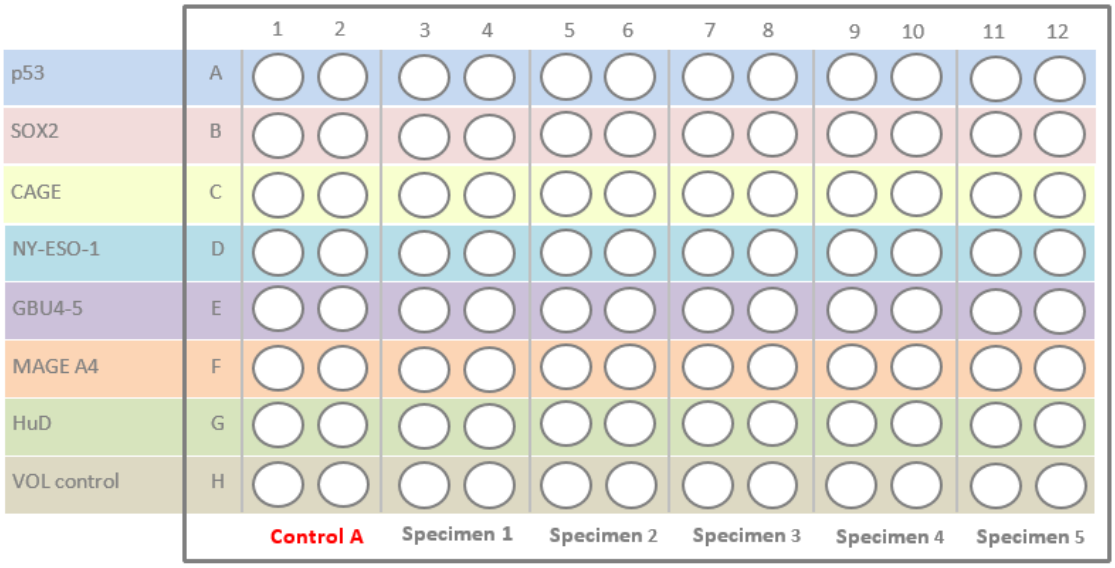

Plate 1 layout when both microtitre plates supplied with a single kit are used at the same time.

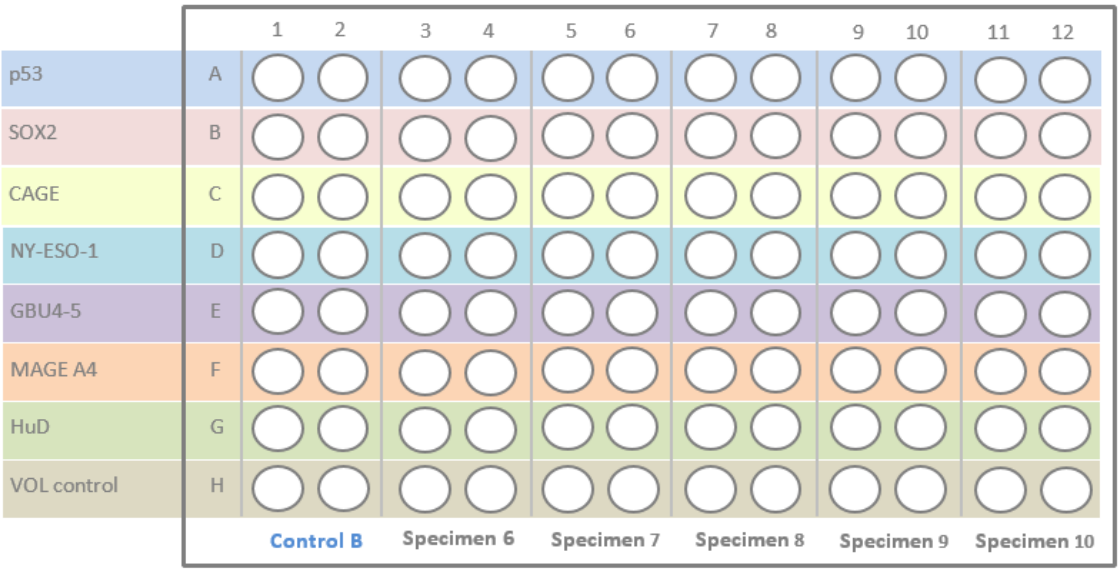

Plate 2 layout when both microtitre plates supplied with a single kit are used at the same time.

c) Once all the control reagent and patient specimens have been added to the microtitre plate(s), cover each plate using a sealing film (Part Number: ECDTL2-SEAL), taking care to ensure the sealing film adequately covers all wells on the plate.
d) Place the covered plate on a shaker at 400rpm and incubate for a total of 90 minutes (as per timer already started) at +18-22°C.
    - If your plate shaker does not have a shaker speed setting, use a 'moderate' shaking speed. Refer to the video demonstrations for an illustration of shaking speed.

Step 3: Secondary antibody addition

a) Carefully remove the sealing film from each plate and empty the well contents into a suitable biological waste container. Tap onto absorbent paper to remove all liquid.
b) Using a dispensing bottle, fill every well with prepared wash buffer and then empty into a suitable biological waste container. Firmly tap the plate onto absorbent paper to remove all liquid from the wells. REPEAT this entire wash step a further three times.
    - If using an automated plate washer, carry-out a four cycle wash (300µL/well) using the wash buffer supplied with the kit.
c) Using an 8-channel pipette, dispense 100µL of secondary antibody working solution into every well of the plate, dispensing into each column sequentially (i.e., 1, 2, 3, 4…etc.).
d) As soon as one of the plates has been filled, start a timer for 60min.
e) Cover each plate and place on a shaker at 400rpm for 60min (as per timer already started) at 18-22°C.

Step 4: Substrate addition

⚠ Read Important Notes below associated with this step.

a) Carefully remove the sealing film from each plate and empty the well contents into a suitable biological waste container. Tap onto absorbent paper to remove all liquid.
b) Using a dispensing bottle, fill every well with prepared wash buffer and then empty into a suitable biological waste container. Firmly tap the plate onto absorbent paper to remove all liquid from the wells. REPEAT this entire wash step a further three times.
    - If using an automated plate washer, carry-out a four cycle wash (300µL/well) using the wash buffer supplied with the kit.
c) Using an 8-channel pipette, dispense 100µL of substrate (Part Number: ECDTL2-SUB) into every well of the plate, dispensing into each column sequentially (i.e., 1, 2, 3, 4…etc.).
d) **Immediately** after the substrate has been dispensed into all 96 wells of the first plate, start a time for 15 minutes and leave the plate to incubate at 18-22°C in the dark.
e) Throughout the 15 minute incubation, tap the side of the plate(s) to ensure the colour that has developed within each well is homogenous, i.e. no clumping is visible.

⚠ **Step 4 - Important Notes:**

- 1 of 2: It is very important that the substrate is not left to incubate for longer than 15 minutes.
- 2 of 2: Ensure the plate is not exposed to light during its 15 minute incubation at 18-22°C.

Step 5: Stop solution addition

a) Add 100µL of Stop Solution (Part Number: ECDTL2-SS) to every well, in the same order as the Substrate was added.
b) The microtitre plate can now be left for a maximum of 30 minutes at +18-22°C before reading.
   - Note that there is no visible colour change when the stop solution is added.
c) Proceed to Step 6.

Step 6: Spectrophotometric measurement

⚠ Read Important Notes below associated with this step.

a) Insert the first microtitre plate into a plate reader, ensuring well A1 is placed in the top left corner.
b) Measure the optical density of each microtitre plate well spectrophotometrically at a wavelength of 650nm.
c) Export the outputted optical density (OD) values to an Excel file for test result calculation.

⚠ **Step 6 - Important Notes:**

- 1 of 2: Do not read the plate using any wavelength other than 650nm
- 2 of 2: If a reference wavelength is required, we recommend using 480nm

Step 7: Test result calculation

⚠ Read Important Notes below associated with this step.

a) Insert the USB device (Part Number: ECDTL2-USB) into a PC and open the file '2 Plate Assay' or '1 Plate Assay' depending on how many plates were run.
b) Enter the kit LOT number into the field 'Enter kit batch' within the software (see figure 4(A)).
c) Enter an ID for each specimen in the appropriate field (see figure 4(B)).
d) Within the 'Data Input' tab, input the OD values from the Excel file generated in step 6 into the appropriate fields (see figure 4(C))
e) Select the 'Results' tab to see the kit test result. For result interpretation, see section 'Interpretation of Results', table 4.

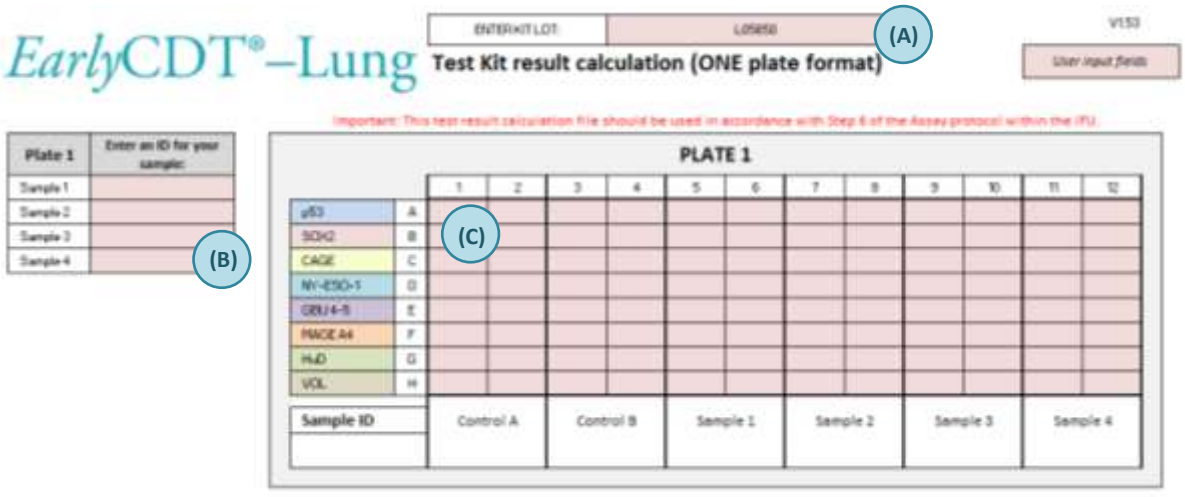

Figure 4. The opening page of the EarlyCDT-Lung Test Kit result calculation software. (A) The correct kit LOT number must be entered in order to use the software. (B) Specimen IDs should be recorded. (C) Exported OD data is entered into the relevant fields.

Step 7 - Important Notes:

- 1 of 1: The software loaded onto the USB device is specific to each LOT of EarlyCDT-Lung Test Kits. Therefore, you must ensure that the USB device that came with the kit is used for result calculation. Failure to use the correct software will give an incorrect test result. The kit LOT number is stated on the kit's outer box label.

Interpretation of Results

The EarlyCDT-Lung Test Kit is designed for professional use and analytical results should be interpreted by skilled medical professionals in combination with all other available clinical information. A moderate or high result indicates that the patient's risk of having lung cancer is greater than that predicted by their gender, age, smoking history and other risk factors alone. This increased risk may warrant a recommendation for additional testing, which could include CT imaging. Table 4 provides a description test results and terms that may be displayed within the kit software.

Table 4: Test result description of terms used within the kit software	
QC acceptance range The Controls used with the kit have an acceptance range which is defined within the 'Lot Specific Insert' included on the USB device supplied with the kit.	
Control Result	**Description**
QC PASSED	Control data falls within the acceptance range.
QC FAILED	Control data falls outside of the acceptance range and the test must be repeated. This may be due to a variety of reasons. Please contact Oncimmune for technical support on earlyCDT@oncimmune.co.uk
Test Result	**Description**
HIGH	One or more autoantibodies in the EarlyCDT-Lung panel are above the high cut-off value.
MODERATE	One or more autoantibodies in the EarlyCDT-Lung panel are above the low cut-off value but all are below the high cut-off value.
NO SIGNIFICANT LEVEL OF AUTOANTIBODIES DETECETED	All autoantibodies in the EarlyCDT-Lung panel are below the low cut-off value. **Note:** A test result of 'No significant level of autoantibodies detected' indicates a lower likelihood of lung cancer than a positive result, however it does not mean that the patient does not have, or will not develop lung cancer. This is because in order to be eligible for the test the patient was already at an elevated risk of lung cancer as predicted by age, gender, smoking history and other risk factors. This has not changed appreciably.
INVALID	If a plate or particular specimen has failed QC checks, then the test result for the respective patient specimen(s) will be "Invalid" and the EarlyCDT-Lung Test should be repeated for those specimen(s). Please contact Oncimmune for technical support on earlyCDT@oncimmune.co.uk
For further information on interpretation of EarlyCDT-Lung test results refer to: http://oncimmune.com/test-results-general/ For further information on the Risk Assessment of Indeterminate Pulmonary Nodules, refer to: http://oncimmune.com/FAQ-nodule	

Summary of Clinical Study Results

The EarlyCDT-Lung test was clinically validated on two separate case control studies. The first study involved three separate patient groups totalling 655 lung cancer patients and 655 normal controls.[3] Cases and controls were matched according to age, gender and smoking history. Clinical sensitivity and specificity of the current 7 autoantibody test has been shown to be 41% and 93% respectively with 92% accuracy.[4] The positive predictive value for a 2.4% prevalence group was calculated to be 1 in 8.[4]

A second study involving 4 separate patient groups totalling 574 lung cancer patients and 678 normal controls matched as before confirmed these findings.[12]

In an audit of clinical outcomes of 1,613 US patients at high risk for lung cancer, whose physician ordered the EarlyCDT-Lung test, performance (sensitivity, specificity and overall accuracy) was consistent with validation data. In the prevalence round >50% of cancers detected by a positive test were early stage disease.[13]

A separate audit of the same patient cohort identified 269 patients with non-calcified pulmonary nodules identified by a radiologist within 6 months of taking the EarlyCDT-Lung test. Of this group 52 patients were found to have lung cancer while the nodules identified in the other 217 patients were benign. Overall, a positive EarlyCDT-Lung test was associated with a 2.2-fold increased risk of lung cancer and for patients with nodules in the 4 to 20mm size range (those that are more likely to be cured), relative risk of lung cancer was 2.7-fold.[14]

The EarlyCDT-Lung Test Kit was validated versus the EarlyCDT-Lung laboratory developed test (LDT) results by running 236 cancers/236 normals, split into Training sets (154 cancers/154 normals) and Validation sets (82 cancers/82 normals). The EarlyCDT-Lung kit performed in a very similar manner to the EarlyCDT-Lung LDT test showing >90% concordance (data on file with Oncimmune).

Assay Performance Characteristics

Reportable Range

The reportable range is defined as the region between the lower and upper limits of quantitation, within which the precision of the calibrated values is acceptable (≤ 20% CV prediction precision on average over all autoantibodies). Analysis of 20 replicate calibration curves has shown that this level corresponds to approximately a range of 7.5% and 92.5% of the calibrator curve maximum. Test results outside this range are recorded as being below or above the limit of quantitation. Such values should still be assessed for the test result and are reported as 'No Significate Level of Autoantibodies Detected' or 'High' respectively.

Precision

To determine intra-assay (within-day) and inter-assay (between-day) precision, 7 serum specimens known to contain positive autoantibody signals were assayed 6 times on the same plate with each plate being run twice a day (separate kits) on 6 separate days. Coefficients of variation (CV) for intra-assay precision, based on the variation between all replicates on the same day, and pooled over all plates over the six days, are shown in Table 5.

Table 5: Intra-assay precision				
Autoantibody	**50nM**		**160nM**	
	Mean OD	**CV%**	**Mean OD**	**CV%**
p53	1.116	10.3	1.735	3.1
SOX2	2.443	3.1	2.782	2.4
CAGE	2.133	4.9	1.568	11.7
NY-ESO-1	3.046	1.4	3.039	1.3
GBU4-5	1.133	7.9	1.462	3.4
MAGE A4	1.418	4.0	1.723	2.1
HuD	1.352	12.9	2.073	2.4

Coefficients of variation (CV) for inter-assay precision, based on the variation between replicates on different days, are shown in Table 6.

Table 6: Inter-assay precision				
Autoantibody	**50nM**		**160nM**	
	Mean OD	**CV%**	**Mean OD**	**CV%**
p53	1.116	13.1	1.735	4.2
SOX2	2.443	3.8	2.782	3.1
CAGE	2.133	5.1	1.568	12.4
NY-ESO-1	3.046	3.3	3.039	3.6
GBU4-5	1.133	11.5	1.462	5.1
MAGE A4	1.418	5.6	1.723	3.3
HuD	1.352	12.9	2.073	4.5

Analytical Sensitivity

Since an independent reference material of known autoantibody concentration is not available for this assay, it is not possible to assess analytical sensitivity.

Linearity

Specimens known to contain high levels of autoantibodies specific for one or more of the EarlyCDT-Lung panel antigens were serially diluted in assay buffer and assayed using the EarlyCDT-Lung protocol described above. Assuming the lowest dilution of the specimens to be 100%, predicted specimen dilution was plotted against known dilution. Slope and correlation coefficients (R^2) for representative signal specimen are given in Table 7.

Table 7: Linearity of the EarlyCDT-Lung Test Through Dilution							
Auto-antibody	**Plate**	**Antigen at 50nM**			**Antigen at 160nM**		
		Intercept	**Gradient**	**R^2**	**Intercept**	**Gradient**	**R^2**
p53	Plate 1	-0.049	0.998	0.979	-0.033	1.000	0.991
p53	Plate 2	-0.036	1.018	0.994	0.030	1.015	0.987
SOX2	Plate 1	-0.040	1.001	0.987	-0.024	1.002	0.996
SOX2	Plate 2	-0.037	1.008	0.991	-0.022	1.004	0.997
CAGE	Plate 1	-0.061	1.000	0.969	0.024	0.962	0.997
CAGE	Plate 2	-0.059	0.996	0.969	0.048	0.940	0.992
NY-ESO-1	Plate 1	-0.066	0.995	0.962	-0.094	0.998	0.931
NY-ESO-1	Plate 2	-0.061	1.003	0.971	-0.100	0.998	0.923
GBU4-5	Plate 1	0.036	1.002	0.977	0.065	1.019	0.951
GBU4-5	Plate 2	0.004	1.006	0.999	-0.047	0.996	0.980
MAGE A4	Plate 1	-0.113	1.016	0.920	-0.115	1.008	0.911
MAGE A4	Plate 2	-0.106	1.023	0.935	-0.109	1.014	0.924
HuD	Plate 1	0.005	0.993	1.000	-0.022	0.995	0.994
HuD	Plate 2	-0.005	0.978	0.995	-0.015	0.996	0.997

Interferences

The effect of potential interfering substances in serum samples positive for autoantibodies measured in the EarlyCDT-Lung Test Kit was evaluated. The following interferents tested (table 8) did not affect the performance of the assay, unless stated otherwise.

Table 8: Interferents tested on the EarlyCDT-Lung Test Kit		
Interferent	**Interferent test concentration***	**Result**
Triglycerides	3274 mg/dL	No significant effect p<0.01
Total protein	120g/L	Significant effect***
Bilirubin (conjugated)	20 mg/dL	No significant effect p<0.01
Bilirubin (unconjugated)	20 mg/dL	No significant effect p<0.01
Haemoglobin	500 mg/dL	No significant effect p<0.01
Albuterol	0.4 µg/mL	No significant effect p<0.01
Digoxin	6.09 ng/mL	No significant effect p<0.01
Rheumatoid factor	1:1**	No significant effect p<0.01
Human anti-mouse antibodies	1:1**	No significant effect p<0.01

*Interferent test concentrations are those recommended by CLSI EP7-A2[15].

** An equal volume of serum positive for either RF or HAMA was added to serum positive autoantibodies measured in the EarlyCDT-Lung Test Kit.

*** The EarlyCDT-Lung Test Kit should not be used in patients known to have diseases that result in an increased level of serum total protein, for example myeloma, amyloidosis, monoclonal gammopathy of undetermined significance (MGUS).

Method Summary

- Prepare reagents
- Dispense 100µL of each relevant control and diluted patient specimen onto the microtitre plate(s) as described.
- Cover and incubate at +18-22°C for 90 minutes with shaking (400rpm).
- Wash 4 times.
- Add 100µL of secondary antibody to all wells of the microtitre plate.
- Cover and incubate at +18-22°C for 60 minutes with shaking (400rpm).
- Wash 4 times.
- Add 100µL of substrate to all wells of the microtitre plate.
- Incubate at +18-22°C in the dark for exactly 15 minutes (no shaking).
- Add 100µL of stop solution to all wells of the microtitre plate.
- Determine the optical density of each well at a wavelength of 650nm within 30 minutes.
- Calculate autoantibody values using the software provided on the USB device.

Glossary of Terms for EarlyCDT-Lung

Accuracy	The degree to which the result of a measurement, calculation, or specification conforms to the correct value or result.
Antigen	Immunogenic protein or other molecule.
Autoantibody (AAb)	Antibody produced in response to a host antigen.
COPD	Chronic obstructive pulmonary disease.
ELISA	Enzyme Linked Immuno-Sorbent Assay.
OD	Optical Density as measured spectrophotometrically.
Pack year	Twenty cigarettes (i.e. one pack) smoked every day for one year.
PBS	Phosphate buffered saline.
Positive Predictive Value	The probability that subjects with a positive test result truly have the disease.
Prevalence	The proportion of a population found to have a disease.
Sensitivity	The proportion of people known to have a disease state, who test positive for it.
Specificity	The proportion of healthy patients known not to have a disease state, who will have a test result of no significant level of autoantibodies detected for it.

Explanation of Symbols

Manufacturer
Indicates the medical device manufacturer.

***In vitro* diagnostic medical device**
Indicates a medical device that is intended to be used as an *in vitro* diagnostic medical device.

Consult instructions for use
Indicates the need for the user to consult the instructions for use.

Biological risks
Indicates that there are potential biological risks associated with the medical device.

Use-by date
Indicates the date after which the medical device is not to be used.

Batch code
Indicates the manufacturer's batch code so that the batch or lot can be identified.

Catalogue Number
Indicates the manufacturer's catalogue number so that the medical device can be identified.

Do not re-use
Indicates a medical device that is intended for one use, or for use on a single patient during a single procedure.

Temperature limit
Indicates the temperature limits to which the medical device can be safely exposed.

Contains sufficient for <*n*> tests
Indicates the total number of IVD tests that can be performed with the IVD kit reagents.

References

1. Stewart BW, Wild CP (Eds.), World Cancer Report 2014, WHO Press, Lyon.
2. The National Lung Screening Trial Research Team, N Engl J Med 2011; 365: 395-409.
3. Boyle P et al., Ann Oncol 2011; 22: 383-9.
4. Chapman CJ et al., Tumor Biol 2012; 33: 1319-26.
5. Chapman CJ et al., Thorax 2007; 63, 228-233.
6. Desmetz C et al., J Cell Mol Med 2011; 15, 2013-2024.
7. Tan EM. J Clin Invest 2001; 108, 1411-1415.
8. Kazarian M & Laird-Offringa IA. Mol Cancer 2011; 10, 33.
9. Zhong L et al., J Thor Oncol 2006; 1, 513–519.
10. Trivers GE et al., Clin Cancer Res 1996; 2, 1767 – 1775.
11. Chapman CJ et al., Clin Cancer Res 2010; 17, 1474-80.
12. Lam S et al., Cancer Prev Res 2011; 4: 1126–34.

13. Jett JR et al., Lung Cancer 2013; 83: 51-5.
14. Massion PP et al., J Thor Oncol 2016; doi.org/10.1016/j.jtho.2016.08.143
15. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition. CLSI document EP7-A2 (ISBN 1-56238-584-4).

Warranty

This product is warranted to perform as described in its labelling and literature when used in accordance with all instructions. Oncimmune Ltd. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Oncimmune Ltd. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser.

Technical Assistance and Customer Service

For technical assistance or to place an order, please telephone Oncimmune on +44 (0)115 82 31869, email earlycdt@oncimmune.co.uk or see our website at www.oncimmune.co.uk

This product is developed and manufactured by:

Oncimmune Ltd.,
Hucknall Road,
Nottingham,
NG5 1PB, UK.