Once developed, the completed assay plates remain stable and they do not therefore need to be read immediately. The plates may be archived for retrospective quality control or re-examination for up to 12 months if kept in a dry, dark environment at room temperature.

QUALITY CONTROL

A typical result would be expected to have few or no spots in the Nil Control and 20 or more spots in the Positive Control (see Figures 4a & b for typical results from the US clinical study).

High numbers of spots in the Nil Control may occur. In addition, high background staining in one or more wells may occur which makes counting of spots difficult. If high background staining occurs such that discrimination of the spots from the background is hindered, the results should be considered invalid. These results are usually due to operator issues, such as suboptimal plate washing, medium contamination or inappropriate specimen handling and PBMC separation methods. It is, however, possible that the state of health of the patient may produce this effect in a small number of cases.

A Nil Control spot count in excess of 10 spots should be considered as 'Invalid'.

Typically, the cell functionality Positive Control spot count should be ≥ 20 or show saturation (too many spots to count). A small proportion of patients may have T cells which show only a limited response to PHA[11]. Where the Positive Control spot count is < 20 spots, it should be considered as 'Invalid', unless either Panel A or Panel B are 'Positive' or 'Borderline (equivocal)' as described in the Results Interpretation and Assay Criteria (see below), in which case the result is valid.

In the case of Invalid results, these should be reported as "Invalid" and it is recommended to collect a further sample and re-test the individual.

RESULTS INTERPRETATION AND ASSAY CRITERIA

Refer to the Quality Control section before applying the following criteria.

NOTE: Diagnosing or excluding tuberculosis disease, and assessing the probability of LTBI, requires a combination of epidemiological, historical, medical and diagnostic findings that should be taken into account when interpreting the T-SPOT.*TB* test. Refer to the most recent CDC guidance (http://www.cdc.gov/tb) for detailed recommendations about diagnosing TB infection (including disease) and selecting persons for testing.

Results for the T-SPOT.*TB* test are interpreted by subtracting the spot count in the Nil control well from the spot count in each of the Panels, according to the following algorithm:

- The test result is Positive if (Panel A-Nil) and/or (Panel B-Nil) ≥ 8 spots.
- The test result is Negative if both (Panel A-Nil) and (Panel B-Nil) ≤ 4 spots. This includes values less than zero.
- Results where the highest of the Panel A or Panel B spot count is such that the (Panel minus Nil) spot count is 5, 6 or 7 spots should be considered Borderline (equivocal) and retesting by collecting another patient specimen is recommended.
- If the result is still Borderline (equivocal) on retesting with another specimen, then other diagnostic tests and/or epidemiologic information should be used to help determine TB infection status of the patient.

The interpretation algorithm is described in the following Flow Diagram (Figure 3) and Tables 1-3. This algorithm also includes quality control criteria.

Figure 3 *– Algorithm Flow Diagram*

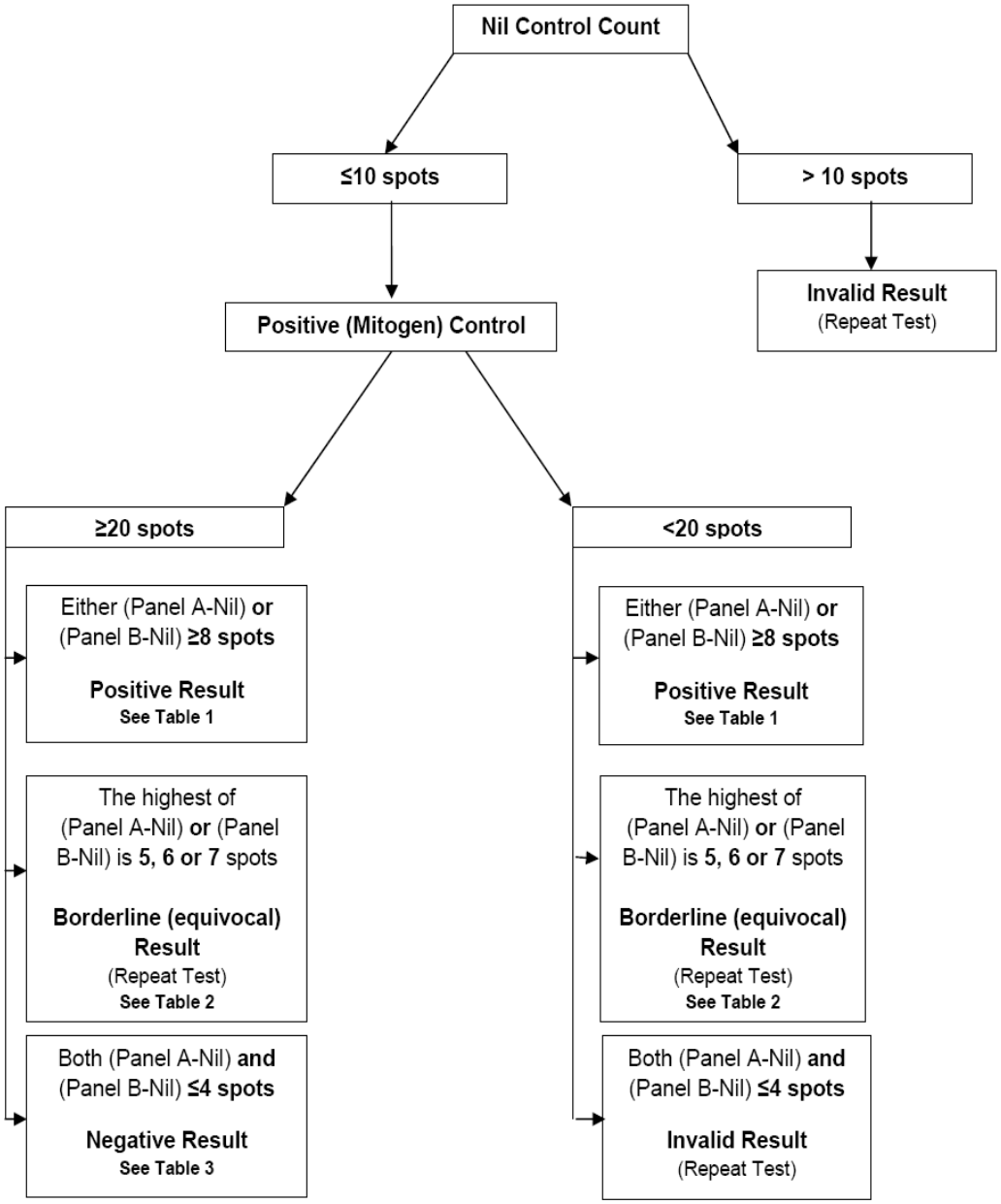

Table 1: *Positive Interpretation: Either (Panel A-Nil) or (Panel B-Nil) ≥8 spots*

Nil Control Well Count	Either Panel A **or** Panel B has the following number of spots[†]	Result Interpretation
0	≥8	**Positive**
1	≥9	**Positive**
2	≥10	**Positive**
3	≥11	**Positive**
4	≥12	**Positive**
5	≥13	**Positive**
6	≥14	**Positive**
7	≥15	**Positive**
8	≥16	**Positive**
9	≥17	**Positive**
10	≥18	**Positive**
>10 spots	n/a	**Invalid****

[†]*Note: The highest Panel-Nil spot count is to be used to determine the test outcome.*

Table 2: *Borderline (equivocal) Interpretation: The highest of (Panel A-Nil) or (Panel B-Nil) is 5, 6 or 7 spots*

Nil Control Well Count	The highest of Panel A **or** Panel B has the following number of spots	Result Interpretation
0	5, 6, or 7	**Borderline (equivocal)***
1	6, 7, or 8	**Borderline (equivocal)***
2	7, 8, or 9	**Borderline (equivocal)***
3	8, 9, or 10	**Borderline (equivocal)***
4	9, 10, or 11	**Borderline (equivocal)***
5	10, 11, or 12	**Borderline (equivocal)***
6	11, 12, or 13	**Borderline (equivocal)***
7	12, 13, or 14	**Borderline (equivocal)***
8	13, 14, or 15	**Borderline (equivocal)***
9	14, 15, or 16	**Borderline (equivocal)***
10	15, 16, or 17	**Borderline (equivocal)***
>10 spots	n/a	**Invalid****

Table 3: *Negative Interpretation: Both (Panel A-Nil) and (Panel B-Nil) ≤4 spots*

Nil Control Well Count	Both Panel A and Panel B has the following number of spots	Result Interpretation
0	≤4	**Negative**
1	≤5	**Negative**
2	≤6	**Negative**
3	≤7	**Negative**
4	≤8	**Negative**
5	≤9	**Negative**
6	≤10	**Negative**
7	≤11	**Negative**
8	≤12	**Negative**
9	≤13	**Negative**
10	≤14	**Negative**
>10 spots	n/a	**Invalid****

** Results where the highest of the Panel A or Panel B spot count is such that the (Panel minus Nil) spot count is 5,6 or 7 spots should be considered Borderline (equivocal) and retesting by collecting another patient specimen is recommended.*
*** In the case of Invalid results, these should be reported as "Invalid" and it is recommended to collect another sample and re-test the individual.*