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Premarket Registration Full Evaluation Route
Summary Assessment Report for
Mikrogen recomLine Treponema IgG

Listing number	MDPR260305C0005
Date of approval	05/03/2026
Product owner	Mikrogen GmbH
Registrant	SPD SCIENTIFIC PTE LTD
Risk Class	Class D
Intended use / Indications of use	The recomLine Treponema IgG is a qualitative in vitro assay for the detection of IgG antibodies against Treponema pallidum in human serum or plasma (EDTA, heparin, citrate, CPD) that have been found positive or questionable using a treponemal and/or non-treponemal screening test procedure to provide supportive evidence of infection with Treponema pallidum. The assay is used as a confirmation and aid in the diagnosis of syphilis infection and the associated diseases, in combination with other laboratory and clinical findings. It is intended for confirmation of blood samples for blood donor services. The recomLine Treponema IgG can be performed manually or automated by trained professional users in a suitable laboratory.

1. Device description

The recomLine Treponema IgG is a qualitative in vitro assay for the detection of IgG antibodies against Treponema pallidum in human serum or plasma (EDTA, heparin, citrate, CPD) that have been found positive or questionable using a treponemal and/or non-treponemal screening test procedures, providing supportive evidence of infection with Treponema pallidum.

The assay serves as confirmation and aids in the diagnosis of syphilis infection and the associated diseases, in combination with other laboratory and clinical findings. It is intended for confirming blood samples for blood donor services. The recomLine Treponema IgG can be performed manually or through automation by trained professional users in a suitable laboratory.

The recomLine Treponema IgG is a line immunoassay. The recomLine test principle allows the identification of specific antibodies against the individual antigens of Treponema pallidum due to the separate lining up of the individual antigens. The following recombinantly produced antigens are applied in the test: Tp47, TmpA, Tp257 (Gpd), Tp453, Tp17 and Tp15.

Highly purified, recombinant Treponema antigens are fixed on nitrocellulose membrane test strips.

1. The test strips are incubated with the diluted serum or plasma sample, and the specific antibodies bind to the pathogen antigens on the test strips.
2. Unbound antibodies are then washed away.
3. In a second step, the strips are incubated with anti-human immunoglobulin antibodies (IgG), which are coupled to horseradish peroxidase.
4. Unbound conjugate antibodies are then washed away.
5. Specific bound antibodies are detected with a staining reaction catalysed by the peroxidase. In case an antigen-antibody reaction has taken place, a dark band will appear on the strip at the corresponding point

Control bands are located at the top end of the test strip:

a) The reaction control below the strip number for which every serum/plasma sample must show reaction
b) The conjugate controls (IgG) are used to check the antibody class detected. The IgG specific test must show positive reactivity in the IgG control band.
c) 'Cut-off control': The intensity of this band enables an evaluation of the reactivity of the individual antigen bands.

The test strips can be analysed visual or computer-assisted – using the test strip analysis software recomScan.

<u>Assessment of the band intensity</u>

1. Note the date, testing person and time of staining as well as lot and tube number along with the antibody class that was detected in the attached evaluation form.
2. Enter the sample identification numbers in the evaluation form.
3. Now stick the corresponding test strips onto the appropriate fields on the evaluation form using a glue stick. Glue may only be applied to the upper area of the strip and not below control and antigen bands. Align the test strip with the reaction control band along the marked lines. Then use transparent adhesive tape to attach the test strip to the left of the marked lines (do not tape over the reaction control band!). Sticking the entire test strip down flat using glue or tape can lead to changes in colour.
4. Now identify the bands of the developed test strip using the control strip printed on the evaluation form and enter these into the evaluation form. Carry out the evaluation of the intensity of the emergent bands for each corresponding immunoglobulin class separately using Table 1.

Table 1: Assessment of band intensity in relation to the cut-off band

Colour intensity of the bands	**Analysis**
No reaction	-
Very low intensity (less than cut-off band)	+/-
Low intensity (equivalent to cut-off band)	+
Strong intensity (stronger than cut-off band)	++
Very strong intensity	+++

Interpretation of test results
Refer to Table 2 for the test interpretation criteria.

Table 2: Test interpretation

Test result	**Criteria**
Negative	No antigen ≥ cut-off
Borderline	Only one random antigen ≥ cut-off
Positive	At least two random antigens ≥ cut-off

The test kit comes in 2 configurations, containing the following:

- 100 ml wash buffer A (10-fold concentrate), 40 ml chromogenic substrate tetramethylbenzidine, 5 g low-fat milk powder, 2 tubes each with 10 consecutively numbered test strips and 500 µl anti-human IgG conjugate (100-fold concentrate, green cap). This is sufficient for 20 measurements.
- 5 x 100 ml wash buffer A (10-fold concentrate), 5 x 40 ml chromogenic substrate tetramethylbenzidine, 5 x 5 g low-fat milk powder, 10 tubes each with 10 consecutively numbered test strips and 5 x 500 µl anti-human IgG conjugate (100-fold concentrate, green cap). This is sufficient for 100 measurements.

A negative test result for recomLine Treponema cannot exclude an infection with Treponema pallidum. Further sampling and testing should be performed after four weeks with existing, clinical suspicion of infection with Treponema pallidum and negative, serological results. Additional limitations, warnings and precautions can be found in the device labelling.

2. List of standards applied

Standards	**Title**
EN 13641	Elimination or reduction of risk of infection related to in vitro diagnostic reagents
EN ISO 15193	In vitro diagnostic medical devices – Measurement of quantities in samples of biological origin – Requirements for content and presentation of reference measurement procedures
EN ISO 15194	In vitro diagnostic medical devices – Measurement of quantities in samples of biological origin – Requirements for certified reference materials and the content of supporting documentation.
EN ISO 17511	In vitro diagnostic medical devices – Requirements for establishing the metrological traceability of values assigned to calibrators, trueness control materials, and human samples
EN 62366-1	Medical devices – Part 1: Application of usability engineering to medical devices.
ISO 13485	Medical devices – Quality management systems – Requirements for regulatory purposes
ISO 14971	Medical Devices – Application of Risk Management to Medical Devices
EN 13612	Performance evaluation of in vitro diagnostic medical devices
EN ISO 23640	In vitro diagnostic medical devices – Evaluation of stability of in vitro diagnostic reagents
EN ISO 20916	In vitro diagnostic medical device -Clinical performance studies using specimens from human subjects – Good study practice
EN ISO 15223-1	Medical devices -Symbols to be used with information to be supplied by the manufacturer. Part 1: General requirements

EN ISO 18113-1	In vitro diagnostic medical devices – Information supplied by the manufacturer (labelling) – Part 1: terms, definitions, and general requirements
EN ISO 18113-2	In vitro diagnostic medical devices – Information supplied by the manufacturer (labelling) – Part 2: in vitro diagnostic reagents for professional use.

3. Pre-clinical assessment

(i) Stability

Real-time stability studies were completed on three lots stored for a minimum of 18 months at 2-8°C to support a shelf-life claim of 18 months when stored at 2-8°C.

For in-use stability, 1 lot was tested in real-time at 2-8°C with final testing timepoint at 25 months, which supported the in-use stability claim through the stated expiry date (18 months representing the worst-case scenario).

A transport stability study was conducted on one lot of the device to stimulate various transport handling conditions, where the test lot was stored at 2-8°C for 12 months, followed by storage at 30 ± 2°C for 7 days, then cooling to 2-8°C again. The test lot was tested directly after the transport simulation in month 12 and then tested again 15 and 18 months after kit manufacturing. The product shelf-life is not impacted by the shipping simulation.

(ii) IVD specific studies

- Analytical specificity: Interfering substances
  For exogenous substances, a control study with serum-plasma pairs taken at the same time point as serum, heparin-, CPD-, sodium citrate- and EDTA-plasma showed that the mentioned anticoagulants have no impact upon the efficiency of the test.

  For endogenous substances, there was no impact to the efficiency of the test with the following concentration ranges:
  - Triglyceride-rich lipoproteins: 93.75–1500 mg/dL
  - Hemolysate: 62.5–1000 mg/dL
  - Conjugated bilirubin: 0.2–40 mg/dL
  - Unconjugated bilirubin: 0.2–40 mg/dL

  Elevated protein concentration in the sample (> 3000 mg/dL) can lead to false positive results in recomLine Treponema IgG.

- Analytical specificity: Cross-reactivity
  The potential interference of antibodies against other organisms that can cause similar clinical symptoms (Borrelia burgdorferi, HCV, HIV, EBV IgM positive, CMV IgM positive) as well as conditions that show an atypical activity of the immune system (e.g. antinuclear autoimmune antibodies, hypergammaglobulinaemia, pregnancy, rheumatoid factor) have been investigated in control studies. Additionally, samples from patients hospitalized for various reasons (pre-COVID) were analysed. Samples with antibodies against the expression system of the recombinant antigens (E. coli) were tested as well. No significant cross reactivity in the tested sample collective was detected with recomLine Treponema IgG.

- Precision
  Intra-assay variance was determined by repeated testing of samples in a single test run, and the CV (coefficient of variation) was calculated based on the resulting COI (cut-off indices) to be <11% for each antigen separately.

Inter-assay and inter-lot variance were determined by running three samples of varying reactivity across three assay procedures on three days using one lot and by running eight samples of varying reactivity across three assay procedures on three days using three different lots respectively, and concordance based on overall test result and number of reacting antigens was determined. Concordance for both inter-assay and inter-lot variance were 100%.

- Linearity
  The recomLine Treponema IgG is not applicable for quantitative measurement. The band intensity does not correspond to a linear relationship to the concentration of the given analytical parameter.

- Specimen stability
  Specimen stability study and literature review validated the claims that claimed specimens may be stored up to 2 weeks at 2-8°C, or at prolonged storage at -20°C or less and undergo a maximum of 3 freeze/thaw cycles.

4. Clinical assessment

IVD Specific Requirements

Clinical performance

- Diagnostic sensitivity

The sensitivity data was compiled from 4 clinical studies, and the device was determined to have a diagnostic sensitivity of 99.1%.

Treponema positive samples	**recomLine Treponema IgG (n = 464*)**
Positive	443
Borderline	17
Negative	4
Diagnostic sensitivity (95% CI)	**99.1% (98.3% - 99.9%)**

**Treponema positive samples confirmed by at least 2 serological tests (one of which is an enzyme immunoassay) for different antibodies to T. pallidum.*

- Diagnostic specificity

The specificity data was conducted using blood donations and hence, the samples are assumed to be true negatives. Diagnostic specificity was determined to be 99.6%.

Blood Donors	**recomLine Treponema IgG (n = 252)**
Positive	0
Borderline	1
Negative	251
Diagnostic specificity (95% CI)	**99.6% (98.8% – 100%)**

- Seroconversion

Seroconversion Syphilis Seroconversion Panel PSS901 (9-member panel of undiluted, naturally occurring plasma samples, collected in the US over 60 days in 2012 from a 41-year-old female; origin: SeraCare Life Sciences Inc., Milford, USA) was evaluated by Treponema IgG. The results correspond to the state of the art (defined by comparative test systems mentioned below).

Days since first bleed	**CMIA**	**TPPA**	**RPR**	**recomLine Treponema IgG**
0	Negative	Negative	Negative	Negative
5	Negative	Negative	Negative	Negative
10	Negative	Negative	Negative	Negative
13	Negative	Negative	Negative	Negative
31	Negative	Positive	Positive	Negative
45	Positive	Positive	Positive	Borderline

48	Positive	Positive	Positive	Positive
52	Positive	Positive	Positive	Positive
59	Positive	Positive	Positive	Positive

Expected values

- Normal population

Values have been determined in normal population based on literature research data:

Year	recomLine Treponema (prevalence, Germany)
2001-2022	0.0645%
2012-2022	0.0085%
2018-2022	0.0091%

- Affected population

Values have been determined in affected patients represented by a presumed Treponema collective in a clinical routine testing:

recomLine Treponema IgG	Affected population (routine panel)
	IgG (n = 671)
Positive	377
Borderline	16
Negative	278
Prevalence	**58.7%**

Expected values might differ in other regions and depend on the composition of the evaluated panel.

5. Assessment of device quality

The device is manufactured in Germany at Mikrogen GmbH. The manufacturing site conforms to ISO 13485:2016 Quality Management System.

6. Conclusion

The recomLine Treponema IgG demonstrates acceptable safety and performance for its intended use of the detection of IgG antibodies against Treponema pallidum in human serum or plasma (EDTA, heparin, citrate, CPD) that have tested positive or questionable using treponemal and/or non-treponemal screening test procedures to provide supportive evidence of infection with Treponema pallidum.

The benefits of this device outweigh the associated risks, as it provides confirmatory testing for syphilis and confirmation of blood samples for blood donor services. This confirmatory capability is essential for accurate diagnosis and appropriate treatment decisions, particularly in pregnant women where untreated syphilis can cause severe complications, and in blood donor screening to ensure transfusion safety.

All foreseeable risks have been systematically identified and mitigated through appropriate design controls, comprehensive labelling, and detailed user instruction. The preclinical and clinical validation data substantiate the device's safety and effectiveness for the intended patient population and clinical environment.