EUROPEAN COMMISSION

View in the context of the Performance Evaluation Consultation Procedure (PECP)

Expert panels on medical devices and in vitro diagnostic devices (Expamed)

Contents

Scope of this expert view

This scientific view reflects the opinion of independent experts (MDR Article 106.1) on the performance evaluation report (PER) of the manufacturer. The advice is provided in the context of the performance evaluation consultation procedure (PECP), which is an additional element of conformity assessment by notified bodies for specific high-risk *in vitro* diagnostic devices (IVDR Article 48.6).

When making its conformity assessment decision, the notified body is obliged to give due consideration to the opinions expressed in the scientific view of the expert panel, where applicable (Annex IX, Section 4.9 or, as applicable, Annex X, Section 3, point (j)).

For class D devices, the notified body must provide a full justification in the case of divergent views between the notified body and the experts. This justification shall be included in the notification to the competent authority (IVDR Article 50; mechanism for scrutiny of class D devices).

1 ADMINISTRATIVE INFORMATION

Date of reception of the dossier	30/09/2021
Notified Body Number	2797
Internal PECP dossier #	IVD-2021-000004
***In vitro* diagnostic medical device**	This test is intended for the direct detection of Babesia (B. microti, B, duncani, B. divergens, and B. venatorum) DNA and RNA in whole blood samples from individual human donors, including donors of whole blood and blood components, and other living donors.

2 INFORMATION PROVIDED BY THE NOTIFIED BODY

When consulting the IVD expert panel, the notified body provided the below information on the type of device in accordance with MDCG 2021-22.

Intended purpose (P)		
P1	what is detected and/or measured *please specify the analyte(s) or marker(s), e.g. SARS-CoV-2 spike protein, Kel1 (K)*	Babesia (B. microti, B, duncani, B. divergens, and B. venatorum) DNA and RNA
P2	function of the device *e.g. diagnosis, aid to diagnosis, monitoring, determining the infectious load, tissue typing etc*	This test is intended for the direct detection of Babesia (B. microti, B, duncani, B. divergens, and B. venatorum) DNA and RNA in whole blood samples from individual human donors, including donors of whole blood and blood components, and other living donors. This test is also intended for use to screen organ and tissue donors when donor samples are obtained while the donor's heart is still beating.
P3	the specific disorder, condition or risk factor of interest that it is intended to detect, define or differentiate *e.g. hepatitis C infection, exposure to SARS-CoV-2, risk of HIV transmission in blood transfusion etc.*	Babesia transmission in blood transfusion

P4	whether it is automated or not	Automated
P5	whether it is qualitative, semi-quantitative or quantitative	Qualitative
P6	type of specimen(s) *e.g. whole blood, serum, saliva etc*	Whole Blood
P7	where applicable, the testing population *e.g. persons with specific health conditions, persons with specific symptoms, children in a certain age range*	Living donors of whole blood and blood components and other living donors. Organ and tissue donors.
P8	intended user	Trained laboratory professionals who are proficient in using automated platform
Technology (T)		
T1	principle of the assay method or principles of operation of the instrument *e.g. real-time PCR, qualitative PCR, digital PCR, sandwich immunoassay, competitive immunoassay, immunoturbidimetric assay etc.*	Real-time PCR

3 VIEWS OF THE EXPERT PANEL

3.1 Information on panel and sub-group

Date of views	19/11/2021
Expert panel name	IVD expert panel
Sub-group of expert panel	IVD sub-group 2021-4

3.2 Summary of expert panel views

The test is a qualitative *in vitro* nucleic acid screening test (NAT) for the direct detection of *Babesia* (*B. microti*, *B. duncani*, *B. divergens*, and *B. venatorum*) DNA and RNA in whole blood samples from individual human donors, including donors of whole blood and blood components, and other living donors. This test is also intended for use to screen organ and tissue donors when donor samples are obtained while the donor's heart is still beating. Whole blood samples from all donors may be screened as individual samples or in pools comprised of aliquots of individual samples. This test is not intended for use as an aid in the diagnosis of *Babesia* infection. This test is not intended for use on samples of cord blood. This test is not intended for use on cadaveric blood specimens.

The *in vitro* diagnostic medical test is an on-market product, CE-marked, under the *In Vitro* Diagnostic Directive (IVDD) as of December 2019. A new evacuated pre-analytic sample collection tube was developed and used to collect whole blood for use with the test. The tube containing the lysed whole blood is used as the primary tube on the analyzer, on which the universal sample preparation steps will be performed.

Babesia is usually tick-borne but is also transmissible by transfusion. Consensus expert opinions/positions from relevant professional associations suggest that the human babesiosis in Europe is primarily attributed to infections with *B. divergens* and to a lesser extent with *B. venatorum* and *B. microti*. While transmission predominantly occurs through the tick Ixodes ricinus, human infection by blood transfusion in Europe have been documented. Strizova et al. (2020) (DOI: 10.14411/fp.2020.031) reported a case of a 36-year-old man in the Czech Republic who experienced severe polytrauma requiring repetitive blood transfusions.

The benefit/risk assessment of the screening assay would be more sustainable if the approach of the epidemiological questionnaire were used. Some European countries ban donating blood if the person has a tick for 14 days. The positive predictive value will be very low if we search for the parasite in extremely low prevalence environments. False-positive cases have been reported.

We consider the manufacturer's justification acceptable for the approach to gathering the clinical evidence. The literature search was conducted using a sufficiently robust methodology, and the literature summary was adequate, supporting the manufacturer's in-house evidence, protocols, and conclusions from the reports.

The NAT is recognized as an appropriate technology for screening for transmissible agents in blood banks. The systematic introduction of this technology since the 1990s substantially reduced the seroconversion/window period for several pathogens. Thus, the manufacturer's selection of this technology guarantees a lower risk of post-transfusion of *Babesia*'s infection.

Safety warnings and precautions are deemed to be adequate for the materials that make up the kit. The reported performance evidence is suitable for the intended use of the IVD. Clinical evidence consisted of scientific validity, analytical performance and clinical performance. The measurements and analyses were sufficiently acceptable in demonstrating the ability of the *Babesia* NAT to classify the results based on the samples tested correctly.

Acceptance criteria were defined by the manufacturer, as follows: the 2-sided 95% exact confidence interval (CI) for sensitivity for both neat and diluted specimens [redacted]; the lower limit of the clinical specificity CI exceeds the 99.747% lower limit of the two-sided 95% CI criterion for sample sizes ≥ 35,000 blood donations. These values meet the targets admitted in most European blood banks for screening tests. All concentrations were reactive at 2-3 times the limit of detection. Endogenous and exogenous interfering substances must be negative, and spiked samples with interfering substances must be reactive. Evidence provided by the manufacturer met the specified acceptance criteria.

To conclude, the manufacturer presents protocols and reported evidence that follows the terminology and requirements contained in REGULATION (EU) 2017/746. The Performance Evaluation Report presents, with robust support, a NAT suitable for its intended use in the blood bank laboratories and others that fit the intended use of the test.

3.3 Views on the specific reports included in the performance evaluation report (PER)

(IVDR, Annex XIII, Section 1.3.2, first paragraph)

Views of the expert panel on the performance evaluation report of the manufacturer (PER)
1. Expert views on the scientific validity report[1]
The manufacturer has demonstrated the scientific validity of the device measuring the four types of *Babesia*, supplemented with a comprehensive literature review of the peer-reviewed scientific literature using keywords relevant for this project. Consensus expert opinions/positions from relevant professional associations are published in peer-reviewed journals such as "Blood" (impact factor (IF) of 22.113), Transfusion (IF 2020 of 3.157) and Transfusion and Apheresis Science (IF 2020 of 1.764). These articles include the clinical and analytical performance of the device. Two publications reported less favourable findings, publications with controversial findings were included. Scientific validity, including the generally acknowledged state of the art, was evaluated through comparison to devices measuring the same analyte/marker, scientific peer-reviewed literature that contains favourable and unfavourable publications, the consensus from expert opinion/guidelines, and results from a retrospective evaluation of Other Sources of Clinical Performance Data.
2. Expert views on the analytical performance report[2]
The manufacturer demonstrates the analytical performance of the device (pages 161-359) in relation to all the parameters described in Table 1 (page 171). The manufacturer's analytical performance report provides sufficient data for the demonstration of the analytical performance of the device in relation to all analytical performance parameters. All data are available for making decisions if the assay is appropriate for use from the analytical point of view. If some parameters are omitted, provide views on the manufacturer's justification. Analytical performance was assessed using technical performance verification studies. These study results demonstrate that the test is suitable for its intended purpose and detects *Babesia* DNA and RNA with sufficient accuracy and precision. Overall, the evidence provided supports the analytical performance claims of the manufacturer, indicating the IVD is fit for the purpose. The 95% hit rate detection limit (determined using the Probit regression model) study involved whole blood samples, non-pre-lysed *Babesia* culture specimens, and *Babesia*. Results of Probit analysis on LoD data collected with *Babesia* infected red blood cells in human whole blood are [iRBC/mL] 6.1 (95% CI: 5.0 - 7.9), 50.2 (95% CI: 44.2 – 58.8), 25.1 (95% CI: 522.3 – 31.8), and 40.0 (95% CI: 34.1 – 48.7) for *B.microti*, *B.duncani*, *B.divergens*, and *B.venatorum*, respectively. The manufacturer assumes "The assay gave repeatable results independent of operator, reagent lot, tube lot, instrument, day and run.". We assume that the results were reported as systematically true, independently from the previous variables changes. In regard to parameters that were omitted, the limit of quantification, measuring range, and linearity studies were not performed, the manufacturer arguing that the test is qualitative and "The *Babesia* Test is qualitative and only reports the presence or absence of the test target. Since the reported result is not quantitative in nature, studies to determine analytical performance for limit of

[1] Annex XIII, Section 1.2.1 of Regulation (EU) 2017/746 - Demonstration of the scientific validity

[2] Annex XIII, Section 1.2.2 of Regulation (EU) 2017/746 - Demonstration of the analytical performance

quantitation, measuring range, and linearity were not conducted." "Accuracy of Measurement - Trueness" is also not reported. The manufacturer considers that "At the time of assay development there were no certified reference material or certified reference methods available for comparison."
The manufacturer concludes that "Based on the results of the studies, the test shows acceptable performance, demonstrating that the *Babesia* test is suitable for its intended purpose and detects *Babesia* DNA and RNA in whole blood samples with sufficient accuracy and precision."
The variance and percentage of the total variance of cycle threshold (Ct) attributed to site, lot, day, batch, and within-batch were presented. Three concentrations were studied for each *Barbesia*'s type. The within batch component contributed the most variability regardless of panel member concentration, ranging from 56.5% to 97.0%. Only the lowest concentrations of *B. duncani and B. diverge*ns reported lower than 95% true positives.

3. Expert views on the clinical performance report[3]

Dossier does not include prospective or restrospective studies done in Europe.
Release of contaminated blood/organ/tissue could result in infection of the recipient. So, the risk of false negative results ("low clinical sensitivity") is a major concern.
Due to low prevalence of infection in Europe, false positive cases can also be expected.
Clinical sensitivity study used known positive samples (comprised of *Babesia* NAT-positive clinical and contrived samples) to evaluate the clinical sensitivity in undiluted (neat) and simulated pools of six. Clinical sensitivity was calculated separately for each sample dilution level, i.e., neat or 1:6. Known positive testing was performed at three different testing sites.
Total of 203 specimens comprised 131 clinical (*B. microti*) and 72 contrived (18 each of *B. microti*, *B. duncani*, *B. divergens*, and *B. venatorum*) specimens known to be positive for Babesia by NAT, were evaluated in this study. The specimens were validated by an "in-house" NAT test for Babesia using unique primers and probes that differ from those used in the test and tested neat and diluted 1:6 (to simulate mini pools of six). Each specimen was tested once at three sites for a total of 609 neat and 609 diluted samples. The manufacturer appears to consider "NAT-positive for *Babesia*" as a "gold test" for Babesia infection. Otherwise, it would be designated "clinical sensitivity" as "positive agreement".
The clinical sensitivity is 100.0 %, with a two-sided 95% confidence interval of 98.2% - 100% for both neat and diluted specimens. The value hits the target of 100% (page 211). The 2-sided 95% exact CI for sensitivity for both neat and diluted specimens exceeded the [redacted] criteria and thus met the acceptance criteria.
The clinical specificity study was conducted using the *Babesia* investigational assay to test donations collected in the US, beginning in approximately the third quarter of 2019 and continuing at least until a minimum number of donations have been screened for the presence of *Babesia* infection.
Donations were tested in pools of six, and reactive pools will be deconstructed to identify the individual aliquot(s) that triggered the reactive pool. The deconstruction component of this study demonstrated that the 6-sample pooling and resolution testing function of the *Babesia* test on the is capable of identifying *Babesia*-positive specimens in pools of 6 individual specimens.
A total of 168,981 evaluable donations were enrolled in this study and tested as individual donations across external laboratory sites. The clinical specificity was calculated as the percentage of *Babesia* donor status-negative donors who had *Babesia* non-reactive results.

[3] Annex XIII, Section 1.2.3 of Regulation (EU) 2017/746 - Demonstration of the clinical performance

The clinical specificity is 99.9 %, with a two-sided 95% confidence interval of 99.9% - 100%. The clinical specificity acceptance criterion has been met overall and individually by each endemicity category. The lower limit of the clinical specificity exceeds the 99.747% lower limit of the two-sided 95% CI criterion for sample sizes ≥ 35,000 blood donations. The data demonstrate a robust medical value for the test. However its clinical value in European setting has never been tested.

3.4 Views on specific assessment aspects of the performance evaluation report (PER)

(IVDR, Annex XIII, Section 1.3.2, second paragraph)

Views of the expert panel on the specific aspects included in the performance evaluation report of the manufacturer (PER)
1. The justification for the approach taken to gather the clinical evidence
The manufacturer states, "No clinical performance studies have been executed under IVDR that would require referencing under Annex XIII, Section 1.2.3. Demonstration of compliance to Annex XIII, Section 2 is not applicable since the historical clinical data that provide substantial and relevant evidence predate IVDR enactment and therefore serve as Other Sources of Clinical Performance Data." However all historical clinical data were gathered in USA where *B. microntí* in prevailant species. No data from Europe, where *B. divergens* is prevailant, were presented.
2. The literature search methodology, protocol and report
A total of six peer-reviewed papers addressing the transfusion-transmitted *Babesia* infections and the use of *Babesia* NAT for donor screening were selected. All aspects of the review of the assay's performance were addressed adequately. Relevant scientific literature was screened extensively, and publications with less favourable/controverting findings were not found. The inclusion of studies in European Centers could bring additional value.
3. The technology on which the device is based, the intended purpose of the device and any claims made about the device's performance or safety
The device is based on nucleic acid test technology, including nucleic acid extraction and purification followed by PCR amplification and detection by probes labelled with fluorescent dyes. Nucleic acid test technology has been used in blood donor screening to decrease the "window period" between initial infection and antibody detection for over a decade and is considered "state of the art". The use of this technology is fit for purpose. The assay under consideration has no significant innovations and has been in use in the market in Europe since 2019. The manufacturer's literature review reviewing real-life usage indicates no performance or safety concerns.
4. Acceptability of clinical evidence (clinical data and performance evaluation results) against state of the art in medicine
The Scientific Validity Report presented a comparison of the performance of the assay under investigation with a peer assay, as well as the results and summary of the literature search. The clinical evidence provided by the manufacturer was sufficient to determine the suitability of the

assay to safely be utilized for its intended use in individual human donors, including donors of whole blood and blood components and human donors of organs and tissues.

3.5 Overall conclusions and recommendations

Overall conclusions and recommendations on the performance evaluation report
On review of the evidence, all relevant aspects of the IVDR requirements were addressed in a satisfactory manner. The assay under review demonstrated appropriate clinical sensitivity and specificity levels, such as on non-clinical performance evaluation studies. Sufficient evidence was presented on the robustness of the assay. The sample types included in the instructions for use, along with their storage conditions were validated. Detection of all four *Babesia*'s species was demonstrated. The technology used by the manufacturer is estimated as representing "state of the art" and is commonly used in blood donor screening.

3.6 Stakeholder information, where available

Relevant information provided by stakeholders, if applicable[4]
Has the Secretariat provided information from stakeholders?
☐ YES ☒ NO
If yes, please summarise the information and how it was taken into account.

3.7 Divergent positions in case no consensus can be reached

In case no consensus on the views can be achieved[5], please summarise divergent positions
There were no divergent opinions of the Chair, Rapporteurs and Reviewers.

Please indicate how many of the experts of the panel had divergent views
Not applicable

[4] According to Article 106.4 of Regulation (EU) 2017/745, expert panels shall take into account relevant information provided by stakeholders including patients' organisations and healthcare professionals when preparing their scientific opinions.

[5] According to Article 106.12 of Regulation (EU) 2017/745, when adopting its scientific opinion, the members of the expert panels shall use their best endeavour to reach a consensus. If consensus cannot be reached, the expert panels shall decide by a majority of their members, and the scientific opinion shall mention the divergent positions and the grounds on which they are based.