Biotrin
A DiaSorin Group Company

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Report Reference: 13058/2011

Kit Type: Parvovirus B19 IFA

Kit Lot Number: 34KT288

Distributer: Imerlab, Chile.

Customer: Catholic University of Chile.

Customer Issue: The customer has observed poor cell coverage and florescein sticking to the bottom of the wells.

1.0 Introduction.

The customer provided a kit of 34KT288 and two sealed slides (from 34KT288 tested by the customer) for analysis at Biotrin. This report outlines the results of the in-house testing performed with the returned customer kit, the retain kit and Biotrin's interpretation of the customer's tested slides. Biotrin retains kits from all lots manufactured until the end of the stated product shelf-life. This is in accordance with our quality systems.

2.0 Analysis.

2.1 Summary:

The returned slides tested by the customer were examined at Biotrin and good cell number was noted in all wells for both infected and uninfected cells. Speckled non-specific fluorescence (NSF) was noted throughout the wells. A green coating was observed around the edge of the wells. When the retain kit and the returned customer kit were tested at Biotrin good cell coverage was observed for both infected and uninfected cells and the speckled NSF noted with the slides tested by the customer was not evident. No green coating was noted at the edge of the wells. The Instructions for Use booklet (IFU) specifications were met for the kit controls and expected results were obtained with the Quality Control (QC) panel of samples. A diagnosis could be clearly made from all wells tested with the retain kit and customer slides tested at Biotrin. Biotrin confirmed the observations made by the customer with the slides tested by the customer but cannot confirm any deficiency in product performance on testing of 34KT288 at Biotrin.

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2.2 Methods and Results:

2.2.1 Retain Testing and Returned Customer Kit Testing of 34KT288.

Slides from the retain kit and the returned customer kit were tested as per the IFU with the kit controls and QC samples. A crossover was also performed whereby a retain slide was tested with the returned customer kit components and vice versa. *Refer to Tables 1 and 2, Appendix 1.*

2.2.2 Results of Retain Testing and Returned Customer Kit Testing.

Refer to Tables 3-6, Appendix 1.

Specifications were met for the kit controls and the QC samples tested with the retain kit and the returned customer kit of 34KT288 and for all crossover testing performed. Cell coverage was acceptable. No speckled NSF was noted, with a clear diagnosis being made from all wells.

2.2.3 Examination of the Returned Customer Slides.

On examination of the returned customer slides it was observed that cell coverage was acceptable. It was confirmed that speckled NSF was observed throughout the wells. This fluorescence was noted between the cells as well as on the cell surface and was not specific to infected/uninfected cells. On examination of the edge of the wells, a green coating was observed which was less apparent towards the centre of the wells. It was confirmed that the returned customer slides were more difficult to read than that of the slides tested at Biotrin due to the level of NSF observed, but that the morphology of the NSF was not the grape-like structures which indicate a Parvovirus B19 specific response.

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3.0 Conclusion.

- On examination of the returned slides tested by the customer cell number was acceptable, but a high level of speckled NSF was confirmed throughout the wells, on the surface of the cells and between cells. This NSF did make the slides difficult to read but the morphology of the NSF did not indicate a true positive Parvovirus B19 response.

- Biotrin testing of the retain kit of 34KT288 did not confirm the customer observation. A clear diagnosis could be made from all wells, with expected results obtained for the kit controls and QC samples.

- Biotrin testing of the returned customer kit of 34KT288 did not confirm the customer's observation. A clear diagnosis could be made from all wells tested at Biotrin, with expected results obtained for the kit controls and QC samples.

- Biotrin's crossover testing of the retain slide with the customer components and vice versa did not confirm the customer's observation. A clear diagnosis could be made from all wells, with expected results obtained for the kit controls and QC samples.

- On review, 136 kits of this lot have been sold to date and no other issues have been reported by customers concerning this lot.

- Batch 34KT288 is meeting specifications set out in the IFU. Root cause for the customer issue could not be identified. The issue appears to be assay related as it could not be reproduced on testing in Biotrin. Biotrin, however, appreciate the customer's concern that the level of NSF present on the returned tested slides could lead to misdiagnosis if interpreted incorrectly. Therefore, Biotrin offer some recommendations to help the customer in future testing of the Parvovirus B19 IFA slides.

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4.0 Interpretation of Results.

The following is a guide outlined in the Interpretation of Results Section of the IFU:

- A sample is considered to be negative for Parvovirus B19 IgM and IgG antibodies if there are no visible fluorescence of the VP1 aggregates ('Bunch of Grapes' morphology).

- A sample can be considered positive for Parvovirus B19 IgM and IgG antibodies if a positive fluorescent result is obtained at a dilution of > 1:16 and > 1:64, respectively. Positive fluorescence is indicated by staining of the distinct VP1 protein aggregates ('Bunch of Grapes' morphology). It is important to ensure that the morphology of the fluorescence is in aggregates as non-specific fluorescence may occur.

- Cells which do not contain VP1 protein are included in each well to allow comparison of positive and negative cells. These stain blood red with Evans Blue counterstain.

- Some individuals may exhibit an aspecific IgM reaction which is shown by a weak fluorescence with a poorly defined staining pattern at a 1:16 serum dilution. This result should be verified by an alternative method (e.g. EIA, Immunoblot).

- Results from Parvo B19 IFA testing should be confirmed using available clinical information and other detection methods.

5.0 Recommendations.

- It is important to ensure that during the sample incubation step the slides are placed in a moist chamber for 3 hours at 35-39^{0}C. A 3 hour incubation is essential for an accurate determination of an IgM result.
- When testing for Parvo IgM it is essential that an IgG Adsorbant be used for IgG and Rheumatoid factor. Cross reactivity and non-specific IgM binding may occur if no adsorbant is used or if the adsorbant is used incorrectly (too short of incubation time with the sample, sample not centrifuged correctly, pellet disturbed on loading of supernatant to the slide wells).
- The adsorbant used should have a minimum binding capacity of 18mg/ml. A final dilution of 1/16 of sample + adsorbant should be used for testing.
- When loading the slides never touch the well itself with the pipette/dropper tip and load 20µl of sample/control and 40µl of conjugate to each well tested.

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- Mark around each well with wax pencil to avoid cross-contamination.
- Washing is a very important step in the assay. Ensure that the wash used is the wash that is provided by Biotrin with the Parvo B19 IFA kit. Incorrect washing may lead to non-specific binding.
- If the conjugate component is not sufficiently washed from the wells speckles of fluorescence will remain attached the bottom of the wells.
- Never direct the wash stream directly at the wells. Aim the wash stream at the top edge of the slide. Ensure that the wash bottle is not emitting a strong stream of wash that may wash off the cells. Always soak slides face up in the wash solution for 10 mins.
- Ensure the slides are fully dry before the addition of conjugate/mounting media. Fan drying of slides is recommended.
- Ensure Mounting Media is applied to each well before the addition of the cover slip and examine the slides at 400 x magnification as per the IFU.
- If storing stained slides for future inspection seal around the cover slip with clear nail varnish to prevent the fluorescence fading and store at 2-8^{0}C.

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Appendix 1.

Table 1- Slide Layout for Retain kit of 34KT288

(2 slides of retain tested as per this layout-1st slide - retain slide+retain components, 2nd slide - retain slide + customer kit components).

1 Negative Control tested with IgG Conjugate	**2** IgG Positive Control	**3** HQC IgG Sample	**4** NQC IgG Sample	**5** LQC IgM Sample
10 Negative Control tested with IgM Conjugate	**9** IgM Positive Control	**8** LQC IgG Sample	**7** HQC IgM Sample	**6** NQC IgM Sample

Table 2- Slide Layout for Customer Returned kit of 34KT288

(2 slides of customer kit tested as per this layout-1st slide - customer slide + customer kit components, 2nd slide – customer slide + retain components).

1 Negative Control tested with IgG Conjugate	**2** IgG Positive Control	**3** HQC IgG Sample	**4** NQC IgG Sample	**5** LQC IgM Sample
10 Negative Control tested with IgM Conjugate	**9** IgM Positive Control	**8** LQC IgG Sample	**7** HQC IgM Sample	**6** NQC IgM Sample

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Sample ID Key:

Internal quality control samples: HQC = High Quality Control Sample

LQC = Low Quality Control Sample

NQC = Negative Quality Control Sample

Table 3-Immunofluoresence Results from Retain slide of 34KT288 +Retain Components.

Neg	+4	+4	Neg	+1/2
Neg	+4	+2/3	+3/4	Neg

Table 4-Immunofluoresence Results from Retain slide of 34KT288 +Customer kit Components.

Neg	+4	+4	Neg	+1
Neg	+4	+4	+2/3	Neg

Table 5-Immunofluoresence Results from Customer Returned kit slide of 34KT288 + Customer kit Components.

Neg	+4	+4	Neg	+1
Neg	+4	+2/3	+3/4	Neg

Table 6-Immunofluoresence Results from Customer Returned kit slide of 34KT288 +Retain Components.

Neg	+4	+4	Neg	+1
Neg	+3/4	+3/4	+4	Neg