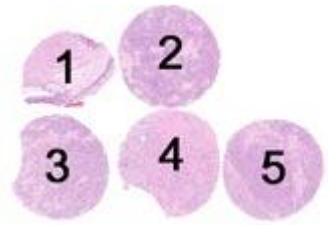
## Assessment Run B12 2011

# Progesterone Receptor (PR)

### Material

The slide to be stained for PR comprised the following five tissues:

| No. | Tissue | PR-positivity* | PR-intensity* |
|---|---|---|---|
| 1. | Uterine cervix | 80-90 % | Moderate to strong |
| 2. | Breast ductal carcinoma | 0 % | Negative |
| 3. | Breast ductal carcinoma | 40 - 60 % | Weak to moderate |
| 4. | Breast ductal carcinoma | 30 - 50 % | Weak to moderate |
| 5. | Breast ductal carcinoma | 80 - 100 % | Moderate to strong |




*PR-status and staining pattern as characterized by NordiQC reference laboratories using the mAb clone 16 (Leica/Novocastra) and the rmAb clone 1E2 (Ventana).

All tissues were fixed in 10% neutral buffered formalin for 24 – 48 hours and processed according to Yaziji et al. (1).

Criteria for assessing a PR staining as optimal included:

- A moderate to strong, distinct nuclear staining reaction of both the columnar and basal squamous epithelial cells and most of the stromal cells (with the exception of endothelial cells and lymphoid cells) in the uterine cervix.
- An at least weak to moderate distinct nuclear staining reaction in the appropriate proportion of the neoplastic cells in the breast ductal carcinomas no. 3, 4 & 5.
- No nuclear staining reaction of the neoplastic cells in the breast carcinoma no. 2 and no more than a weak cytoplasmic reaction in cells with a strong nuclear staining.

For the mAb clones PR 636 and 1A4 a cytoplasmic staining in the columnar epithelial cells and the intermediate squamous epithelial cells, respectively, was accepted.

A staining was classified as good if ≥ 10 % of the neoplastic cells in the breast ductal carcinomas no. 3, 4 & 5 showed an at least weak nuclear staining reaction but below the reference ranges.

A staining was assessed as borderline if ≥ 1 % and < 10 % of the neoplastic cells showed a nuclear staining reaction in one or more of the breast ductal carcinomas no. 3, 4 & 5.

A staining was assessed as poor if < 1% of the neoplastic cells showed a nuclear staining reaction in one or more of the breast ductal carcinomas no. 3, 4 and 5 or a false positive nuclear staining reaction was seen in the breast ductal carcinoma no. 2.

1. Yaziji H, Taylor CR, Goldstein NS, Dabbs DJ, Hammond EH, Hewlett B, Floyd AD, Barry TS, Martin AW, Badve S, Baehner F, Cartun RW, Eisen RN, Swanson PE, Hewitt SM, Vyberg M, Hicks DG; Members of the Standardization Ad-Hoc Consensus Committee. Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry. Appl Immunohistochem Mol Morphol. 2008 Dec;16(6):513-20. PubMed PMID: 18931614.

224 laboratories participated in this assessment. 68 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for PR, run B12**

| Concentrated Abs | N | Vendor | Optimal | Good | Borderl. | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **1A6** | 3<br>1 | Leica/Novocastra<br>Vector | 0 | 4 | 0 | 0 | - | - |
| mAb clone **16** | 21<br>1<br>1 | Leica/Novocastra<br>Monosan<br>Vector | 10 | 5 | 6 | 2 | 65 % | 91 % |
| mAb clone cocktail **16 +** | 2 | Leica/Novocastra | 0 | 1 | 1 | 0 | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **SAN27** | | | | | | | | |
| mAb clone cocktail **hPRa2+hPRa3** | 1 | Thermo/NeoMarkers | 0 | 0 | 0 | 1 | - | - |
| mAb clone **PR 1294** | 3 | Dako | 0 | 2 | 1 | 0 | - | - |
| mAb clone **PR 636** | 52 | Dako | 13 | 14 | 8 | 17 | 52 % | 69 % |
| rmAb clone **RBT22** | 2 | Bio SB | 0 | 0 | 1 | 1 | - | - |
| rmAb clone **SP2** | 4 | Thermo/NeoMarkers | 1 | 0 | 0 | 3 | - | - |
| rmAb clone **SP42** | 1 | Zytomed | 0 | 0 | 1 | 0 | - | - |
| rmAb clone **Y85** | 2<br>1 | Cell Marque<br>Master Diagnostica | 1 | 0 | 2 | 0 | - | - |
| **Ready-To-Use Abs** | | | | | | | | |
| mAb clone **16, PA0312** | 5 | Leica/Novocastra | 1 | 4 | 0 | 0 | 100 % | 100 % |
| mAb clone **16, PM424** | 1 | Biocare | 0 | 1 | 0 | 0 | - | - |
| mAb clone **PR 1294, SK310/K4071** | 5 | Dako | 0 | 0 | 2 | 3 | 0 % | - |
| mAb clone **PR 636, IS/IR068** | 33 | Dako | 15 | 10 | 5 | 3 | 76 % | 85 % |
| mAb clone **PR 636, N1630** | 1 | Dako | 0 | 0 | 1 | 0 | - | - |
| rmAb clone **1E2, 790-223/4296** | 83 | Ventana | 31 | 38 | 11 | 3 | 83 % | 85 % |
| rmAb clone **SP2, ZA0255** | 1 | Zhongshan | 0 | 1 | 0 | 0 | - | - |
| **Total** | 224 | | 72 | 80 | 39 | 33 | - | |
| **Proportion** | | | 32 % | 36 % | 17 % | 15 % | 68 % | |

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

The following central protocol parameters were used to obtain an optimal staining:

**Concentrated Abs**
mAb clone **16**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (1/1)*, Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (2/2)*, Bond Epitope Retrieval Solution 2 (BERS2; Bond, Leica) (5/6)*, BERS1 (Bond, Leica) (1/1)* or EDTA/EGTA pH 8 (1/1)* as the retrieval buffer. The mAb was typically diluted in the range of 1:200 – 1:1,600 depending on the total sensitivity of the protocol employed. Using these protocol settings 10 out of 11 (91 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

mAb clone **PR 636**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (4/11), TRS pH 9 (Dako) (2/11), TRS pH 9 (3-in-1,Dako) (6/11) or BERS2 (Bond, Leica) (1/4) as the retrieval buffer. The mAb was typically diluted in the range of 1:100 – 1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings 20 out of 29 (69 %) laboratories produced a sufficient staining.

rmAb clone **SP2**: The protocol giving an optimal result was based HIER using Tris-EDTA/EGTA pH 9 (1/2) as the retrieval buffer. The rmAb was diluted 1:200.

rmAb clone **Y85**: The protocol giving an optimal result was based on HIER using EDTA/EGTA pH 8 (1/1) as the retrieval buffer. The rmAb was diluted 1:100.

**Ready-To-Use Abs**
mAb, clone **16** (prod.no. PA0312, Leica/Novocastra). The protocol giving an optimal result was based on HIER in BERS2 (Bond, Leica) for 25 min, an incubation time of 15 min in the primary Ab and Refine (DS9800) as the

detection system. Using similar protocol settings 3 out of 3 produced a sufficient staining.

mAb clone **PR 636** (prod. no. IS/IR068, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using TRS pH 9 or TRS pH 9 (3-in-1) (heating time 10-30 min at 95-97°C) , an incubation time of 15-30 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings 23 out of 27 (85 %) laboratories produced a sufficient staining.

rmAb clone **1E2** (prod. no. 790-2223/4296, Ventana): The protocols giving an optimal result were typically based on HIER using mild or standard Cell Conditioning 1, an incubation time of 20-48 min in the primary Ab and iView (760-091) or UltraView (760-500) as the detection system. Using these protocol settings 56 out of 66 (85 %) laboratories produced a sufficient staining (optimal or good).

The most frequent causes of insufficient stains were:
- Too low concentration of the primary Ab
- Less successful primary Ab
- Insufficient epitope retrieval (too short efficient HIER time and/or use of a non-alkaline HIER buffer).

In this assessment and in concordance with the previous PR assessments in NordiQC, the insufficient results were mainly due to a too weak or completely false negative staining. This pattern was seen in 89 % of the insufficient staining results (65 out of 72 stains). Virtually all the laboratories could demonstrate PR in the ductal breast carcinoma no. 5 with 80-100% positivity and a strong nuclear staining intensity (as established by the NordiQC reference laboratories) whereas the prevalent feature of the insufficient staining was a too weak (< 10% positivity) or entirely false negative staining of the ductal breast carcinoma no. 3 and 4 (with 30-60% positivity and a weak to moderate nuclear staining intensity expected). The insufficient staining reactions were typically caused by a too low concentration of the primary Ab and/or insufficient HIER, but also when using less successful Abs with an apparently low PR affinity. However it also has to be mentioned that a high proportion of stains assessed as insufficient (18 out of 72 protocols) seemed to be based on optimal protocol settings, including a correct titre of an otherwise successful primary Ab, HIER settings and detection kit. Thus, the combination of several suboptimal protocol settings might have caused a reduced sensitivity, e.g., a low concentration of the primary Ab, HIER in a non-alkaline buffer and a detection system with only a moderate sensitivity such as a two-step polymer based kit. For the laboratories using a non-alkaline buffer for HIER the overall pass rate was 34 % (13 out of 38), compared to a pass rate of 74 % when HIER was performed with an alkaline buffer. The same pattern was seen when the pass rates were related to the detection system: With a moderately sensitive detection system, such as a 2-step polymer/multimer based detection system or an avidin-biotin based system, an overall pass rate of 62 % was seen (105 out of 169) compared to 86 % (47 out of 55) for a 3-step polymer/multimer based system.
All 4 stains based on the mAb clone PR 636 and performed on the BenchMark platform (Ventana) were assessed as insufficient, despite of protocol settings similar to the settings giving a sufficient result on other IHC platforms, e.g. the Dako Autostainer.

In 6 % of the insufficient results (4 out of 72 stains) a combination of a too weak staining and impaired morphology was seen. This pattern was typically seen by the use of excessive HIER in combination with a protocol giving a too low sensitivity.
In the remaining 3 insufficient stains (5 %) a false positive staining in the PR negative ductal carcinoma no. 2 was seen. When a false positive reaction was observed in the PR negative tumour also a false positive nuclear reaction was seen in scattered non-epithelial cells as lymphocytes and endothelial cells. The false positive staining was seen for the rmAb clone SP2 (2 out of 5 protocols) and the mAb clone cocktail hPRa2+hPRa3 (1 out of 1 protocol). A false positive nuclear reaction was also observed and described in the previous assessments run B6 and B9 and most likely caused by a combination of inadequate washing in buffer and too high concentration of the primary Ab.

For both the mAb clones 16 and PR 636 the Ready-To-use systems from Leica/Novocastra and Dako, respectively, the obtained pass rates were higher than the pass rates obtained for the same clones used with an in-house validated assay.

As observed in the previous assessments for PR, the uterine cervix seems to be an appropriate control for the evaluation of the sensitivity of the PR staining: With an optimal protocol almost all the columnar epithelial cells, the basal squamous epithelial cells and most of the stromal cells must show a strong and distinct nuclear staining with only a minimal cytoplasmic reaction. Virtually all laboratories obtaining this staining pattern were assessed as sufficient. However, differences regarding the reaction pattern are seen depending on the Ab selected. When

using the mAb clone 1A6, the basal squamous epithelial cells are negative and a cytoplasmic reaction is seen in the intermediate and superficial squamous epithelial cells, while the clone PR 636 gives an intense cytoplasmic reaction in the columnar epithelial cells.

This was the 6th assessment of PR in NordiQC. A decrease in the proportion of sufficient results is seen in the two latest runs for PR as shown in table 2:

Table 2. **Proportion of sufficient results for PR in the six NordiQC runs performed**

| | Run 10 2004 | Run B2 2006 | Run B4 2007 | Run B6 2008 | Run B9 2010 | Run B12 2011 |
|---|---|---|---|---|---|---|
| Participants, n= | 54 | 60 | 95 | 111 | 177 | 224 |
| Sufficient results,% | 68 % | 74 % | 80 % | 82 % | 77 % | 68 % |

In the last two assessments for PR many new laboratories have enrolled in the NordiQC programme. In the current run a slightly higher pass rate was observed for the laboratories participating in all three runs from B6 to B12 than for the laboratories participating for the first time in either run B9 or B12: For the laboratories participating for the first time in run B9 or B12 the pass rate was 64 % (86 out of 134), whereas the pass rate was 73 % (66 out of 90 laboratories) for the laboratories participating in all three runs from B6 to B12.

**Conclusion**

The mAb clones 16, PR 636 and the rmAb clone 1E2 are all well performing and recommendable Abs for PR. In general the Ready-To-Use systems for these three Abs showed a superior pass rate compared to in-house validated assays for PR. Efficient HIER (preferably in an alkaline buffer) is mandatory for an optimal performance and must be carried out to provide an optimal balance between sensitivity and morphology. Uterine cervix is an appropriate control, in which almost all the columnar epithelial cells, the basal squamous epithelial cells and most of the stromal cells must show a strong and distinct nuclear staining reaction with only a minimal cytoplasmic staining.

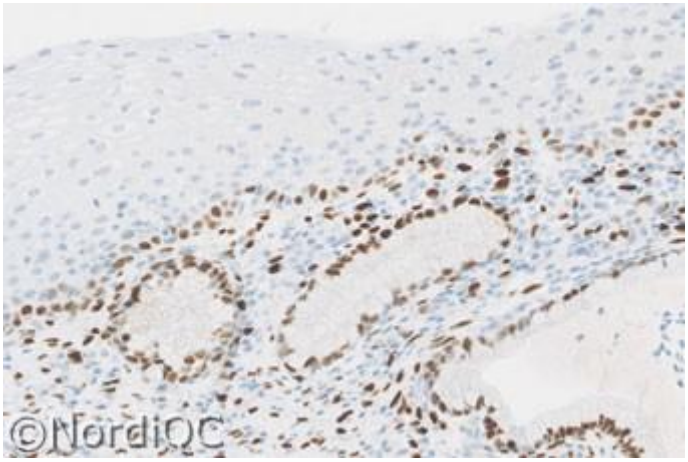



Fig. 1a
Optimal staining for PR of the uterine cervix using the rmAb clone 1E2 as Ready-To-Use and efficient HIER in an alkaline buffer. Virtually all the columnar epithelial cells, the majority of the stromal cells and the basal squamous epithelial cells show a moderate to strong nuclear staining reaction.

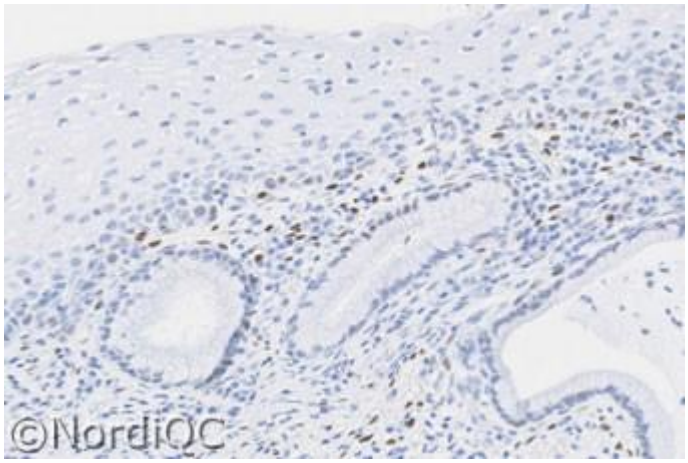



Fig. 1b
Insufficient staining for PR of the uterine cervix, using the mAb clone 16 with protocol settings giving a too low sensitivity (too short HIER in Citrate pH 6) - same field as in Fig. 1a. Scattered stromal cells are demonstrated, but the columnar and basal squamous epithelial cells are virtually negative. Also compare with Figs. 2b & 3b – same protocol.

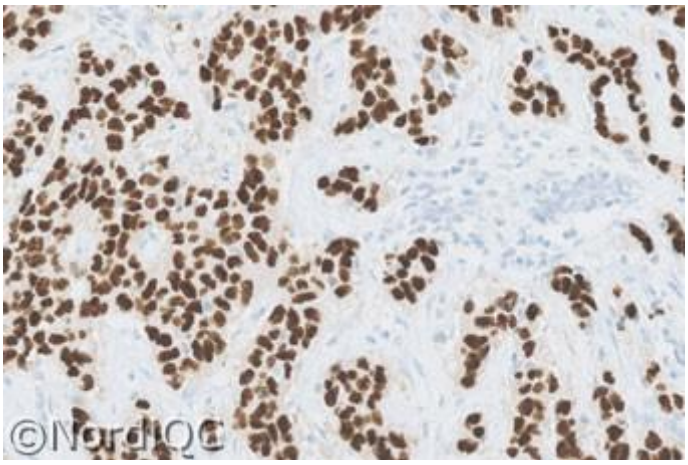



Fig. 2a
Optimal staining for PR of the breast ductal carcinoma no. 5 with 80 - 100 % cells positive using same protocol as in Fig. 1a.
Virtually all the neoplastic cells show a strong and distinct nuclear staining reaction. No background reaction is seen.

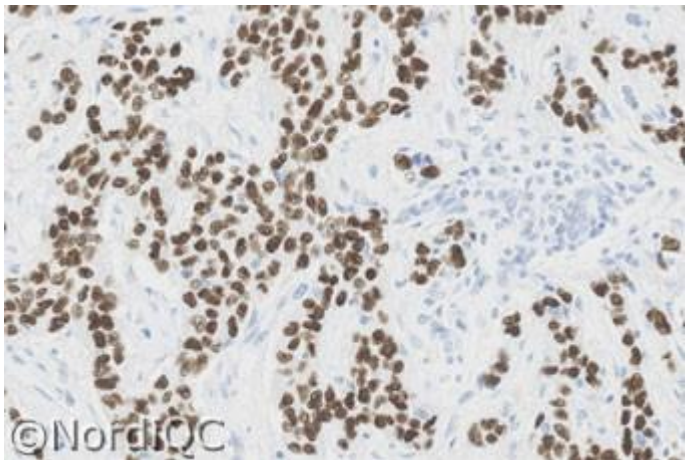



Fig. 2b
Staining for PR of the breast ductal carcinoma no. 5 with 80 - 100 % cells positive using same protocol as in Fig. 1b - same field as in Fig. 2a.
The majority of the neoplastic cells show a moderate and distinct nuclear staining reaction. However also compare with Fig. 3b - same protocol.

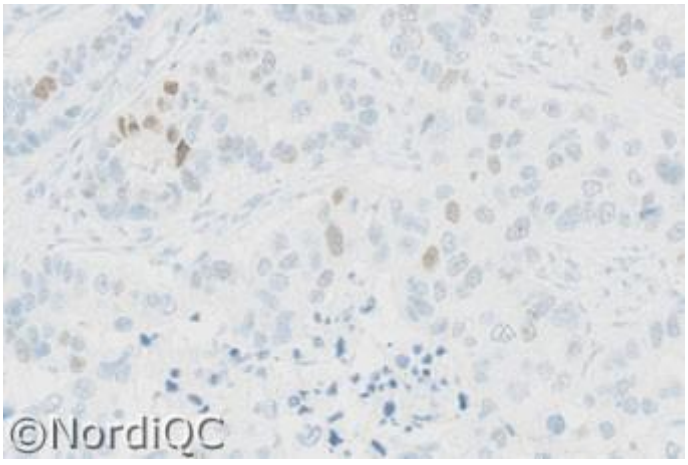



Fig. 3a
Optimal staining for PR of the breast ductal carcinoma no. 4 with 30 - 50 % cells positive using same protocol as in Figs. 1a & 2a.
The PR positive cells are easily recognized, as no cytoplasmic or background reaction is seen.

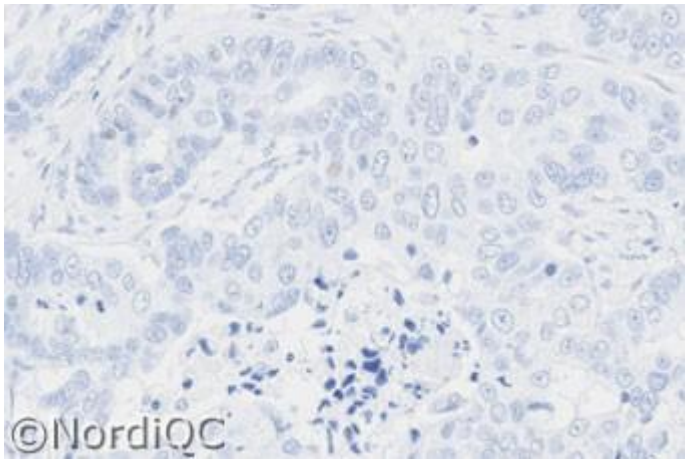



Fig. 3b
Insufficient staining for PR of the breast ductal carcinoma no. 4 with 30 - 50 % cells positive using same protocol as in Figs. 1b and 2b - same field as in Fig. 3a.
Virtually no nuclear staining reaction is seen.

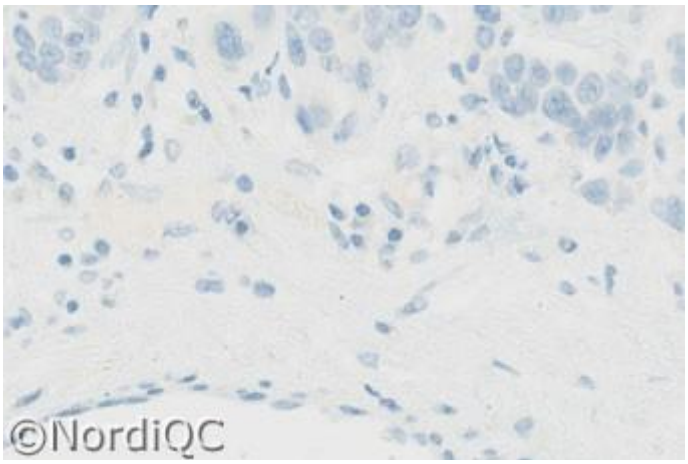



Fig. 4a
Optimal staining of the PR negative breast ductal carcinoma no. 2 using same protocol as in Figs. 1a - 3a.
No nuclear staining reaction is seen in the neoplastic cells. The PR status was tested and confirmed by different Abs and protocol settings in the NordiQC reference laboratories.

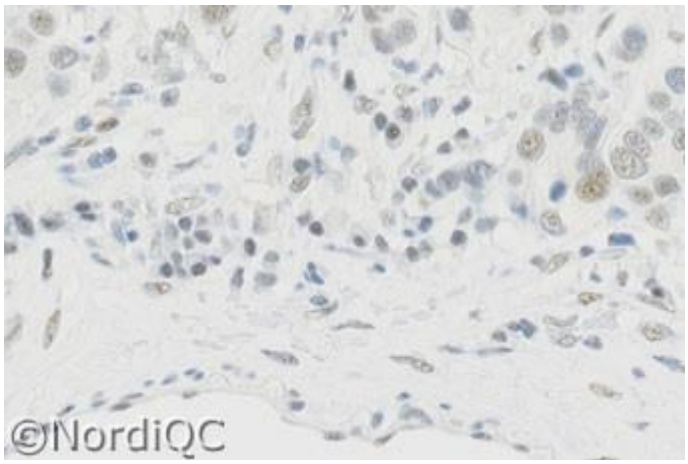



Fig. 4b
Insufficient staining of the PR negative breast ductal carcinoma no. 2 using the rmAb clone SP2.
Both the neoplastic cells, lymphocytes and endothelial cells show a weak and aberrant false positive nuclear staining.
This pattern most likely was caused by the combination of efficient HIER and usage of a too high Ab concentration of the rmAb clone SP2.

SN/MV/LE 6-12-2011