Supplementary information for
‘Results of a worldwide survey on the currently used histopathological diagnostic criteria for invasive lobular breast cancer’ by De Schepper et al.

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### Supplementary Table 1: survey questionnaire

| **In which country do you practice pathology?** |
| **What is your field of interest in pathology?** |
| **In which center do you perform your activities?** |
| **What is the average volume/year of breast cancer samples in your center?** |
| **How many pathologists handle breast cancer specimens in your laboratory?** |
| **Do you perform consensus diagnosis for difficult cases in your center?** |
| **Are you aware of national/international guidelines recommending the use of IHC for the diagnosis of ILC?** |
| **How do you diagnose an invasive lobular carcinoma?** |
| **Which subtypes of ILC do you report? (Multiple options possible):** |
| - Classic |
| - Non-classic |
| - Mixed non-classic |
| - Alveolar |
| - Solid |
| - Trabecular |
| - Histiocytoid/Apocrine |
| - Pleomorphic |
| - Tubulo-lobular |
| - Invasive carcinoma with mixed ductal and lobular features |
| - Mucinous |
| - All of the above |
| - Other |
| **What is the proportion of ILCs diagnosed with ancillary IHC in your institution?** |
| **If you perform IHC, which antibody/antibodies do you use?** |
| - E-cadherin |
| - Beta-catenin |
| - p120 catenin |
| - Other (specify later) |
| - Double staining (please specify) |
| **Which patterns of staining’s do you use to support the diagnosis of ILC by E-cadherin? (multiple options possible)** |
| - Complete absence of membranous E-cadherin staining |
| - Dot-like, perinuclear Golgi-type pattern |
| - Fragmented, focal, or fragmented, focal, or beaded expression beaded expression of E-cadherin |
| - A complete, but weak membranous E-cadherin |
| - All of the above |
| - Other |
| **Do you use upfront E-cadherin IHC for every breast cancer sample?** |
| **When E-cadherin is positive but the H&E pattern is lobular: do you use p120 catenin or / and beta-catenin?** |
| **Which pattern do you consider for ILC diagnosis? (Multiple options possible):** |
| - cytoplasmic or negative beta catenin |
| - cytoplasmic or nuclear p120 staining |
| - Do you use an external control for staining? |
| **If you use E-cadherin, what are its specifications?** |
| Clone: |
| Firm: |
| Concentration: |
### Tissue for validation:

**How do you perform antigen retrieval for E-cadherin?**

If you perform HIER, how is it performed?

Which buffer is used?

At which pH?

If you use enzymatic retrieval, which agent do you use?

If you use beta-catenin, what are its specifications?

Clone:

Firm:

Concentration:

**Tissue for validation:**

**How do you perform antigen retrieval for beta-catenin?**

If you perform HIER, how is it performed?

Which buffer is used?

At which pH?

If you use enzymatic retrieval, which agent do you use?

If you use p120-catenin, what are its specifications?

Clone:

Firm:

Concentration:

**Tissue for validation:**

**How do you perform antigen retrieval for p120-catenin?**

If you perform HIER, how is it performed?

Which buffer is used?

At which pH?

If you use enzymatic retrieval, which agent do you use?

Do you use another antibody other than E-cadherin, beta-catenin and/or p120-catenin?

If you use another antibody, what are its specifications?

Specify antibody:

Clone:

Firm:

Concentration:

**Tissue for validation:**

**How do you perform antigen retrieval for this antibody?**

If you perform HIER, how is it performed?

Which buffer is used?

At which pH?

If you use enzymatic retrieval, which agent do you use?

Do you use DNA sequencing for cases with inconclusive features?
### Supplementary Table 2: characteristics of participants per continent

| Center of activities          | Africa (n (%)) | Asia (n (%)) | Europe (n (%)) | North America (n (%)) | Oceania (n (%)) | South America (n (%)) | p-value |
|------------------------------|----------------|--------------|----------------|-----------------------|----------------|-----------------------|---------|
| **Large tertiary hospital**  | 3 (75.0)       | 11 (40.7)    | 26 (28.3)      | 2 (18.2)              | 1 (50.0)       | 1 (9.1)               | 0.1424  |
| **Private laboratory**       | 0 (0.0)        | 1 (3.7)      | 9 (9.8)        | 0 (0.0)               | 0 (0.0)        | 4 (36.4)              |         |
| **Small community hospital** | 0 (0.0)        | 2 (7.4)      | 4 (4.3)        | 0 (0.0)               | 0 (0.0)        | 1 (9.1)               |         |
| **University Hospital**      | 1 (25.0)       | 13 (48.1)    | 53 (57.6)      | 9 (81.8)              | 1 (50.0)       | 5 (45.5)              |         |
| **Average volume breast cancer samples/year** | | | | | | | |
| 0-150 samples                | 1 (25.0)       | 4 (14.8)     | 1 (1.1)        | 0 (0.0)               | 0 (0.0)        | 2 (18.2)              | 0.0005  |
| 151-300 samples              | 2 (50.0)       | 10 (37.0)    | 15 (16.3)      | 1 (9.1)               | 0 (0.0)        | 1 (9.1)               |         |
| 301-500 samples              | 1 (25.0)       | 3 (11.1)     | 22 (23.9)      | 0 (0.0)               | 2 (100.0)      | 2 (18.2)              |         |
| >500 samples                 | 0 (0.0)        | 10 (37.0)    | 54 (58.7)      | 10 (90.9)             | 0 (0.0)        | 6 (54.5)              |         |
| **Number of pathologist handling breast cancer specimen** | 1 | 0 (0.0) | 7 (25.9) | 1 (1.1) | 0 (0.0) | 0 (0.0) | 2 (18.2) | 0.0005 |
| 1 to 4                       | 0 (0.0)        | 11 (40.7)    | 51 (55.4)      | 4 (36.4)              | 0 (0.0)        | 8 (72.7)              |         |
| 5 or more                    | 4 (100.0)      | 9 (33.3)     | 40 (43.5)      | 7 (63.6)              | 2 (100.0)      | 1 (9.1)               |         |
| **Total**                    | 4              | 27           | 92             | 11                    | 2              | 11                    |         |
**Supplementary Table 3: Overview of used concentration per used E-cadherin clone**

| Concentration per clone | Number of participants |
|-------------------------|------------------------|
| **NCH-38**              |                        |
| RTU                     | 12 (13%)               |
| 1/100                   | 10 (11%)               |
| 1/50                    | 4 (4%)                 |
| 1/200                   | 3 (3%)                 |
| 1/300                   | 1 (1%)                 |
| 1/50 to 1/100           | 1 (1%)                 |
| 1/170                   | 1 (1%)                 |
| 1/25                    | 1 (1%)                 |
| Missing data            | 5 (5%)                 |
| **Clone 36**            | **15 (16%)**           |
| RTU                     | 8 (9%)                 |
| 0.314 µg/ml             | 2 (2%)                 |
| 1/200                   | 1 (1%)                 |
| Missing data            | 4 (4%)                 |
| **EP700Y**              | **14 (15%)**           |
| 1/200                   | 4 (4%)                 |
| RTU                     | 5 (5%)                 |
| 0.314 µg/ml             | 1 (1%)                 |
| 1/700                   | 1 (1%)                 |
| unknown                 | 1 (1%)                 |
| Missing data            | 1 (1%)                 |
| **Clone 36B5**          | **7 (8%)**             |
| RTU                     | 3 (3%)                 |
| 1/100                   | 1 (1%)                 |
| 1/40                    | 1 (1%)                 |
| Missing data            | 2 (2%)                 |
| **unknown**             | **6 (7%)**             |
| Missing data            | 6 (7%)                 |
| **4A2C7**               | **5 (5%)**             |
| 57µg/l                  | 1 (1%)                 |
| 1.25 µg/ml              | 1 (1%)                 |
| 1/50                    | 1 (1%)                 |
| 570µg/l                 | 1 (1%)                 |
| Missing data            | 1 (1%)                 |
| **HECD1**               | **1 (1%)**             |
| Item         | Count |
|-------------|-------|
| 1/50        | 1 (1%)|
| IHC564      | 1 (1%)|
| Unknown     | 1 (1%)|
| EP6         | 1 (1%)|
| 1/1000      | 1 (1%)|
| ECH-6       | 1 (1%)|
| 1/100       | 1 (1%)|
| G-10        | 1 (1%)|
| 1/100       | 1 (1%)|
| GM016       | 1 (1%)|
| RTU         | 1 (1%)|
| Grand Total | 91     |
Supplementary Table 4: E-cadherin antibody clone per continent

| Continent     | Total | Missing data | NCH-38 | EP700Y | Clone 36 | Clone 36B5 | 4A2C7 | ECH-6 | HECD1 | GM016 | G-10 | EP6 | IHC564 |
|---------------|-------|--------------|--------|--------|----------|------------|-------|-------|-------|-------|------|-----|--------|
| Europe        | 92    | 36           | 24     | 11     | 10       | 5          | 2     | 1     | 1     | 1     | 1    | 1   | 1      |
| Asia          | 27    | 8            | 8      | 4      | 3        | 2          |       |       |       |       |      |     |        |
| South America | 11    | 4            | 4      | 2      | 2        | 1          |       |       |       |       |      |     |        |
| North America | 11    | 7            |        | 2      | 1        | 1          |       |       |       |       |      |     |        |
| Africa        | 4     | 2            |        | 1      | 1        |            |       |       |       |       |      |     |        |
| Oceania       | 2     | 1            |        |        |          | 1          |       |       |       |       |      |     |        |
| Grand Total   | 147   |              |        |        |          |            |       |       |       |       |      |     |        |
Supplementary Table 5: staining procedures per antibody

| Epitope retrieval modality          | E-cadherin | β-catenin | p120-catenin |
|-------------------------------------|------------|-----------|--------------|
| Enzymatic retrieval                 | 2          | 1         | 0            |
| Heat induced epitope retrieval (HIER)| 76         | 31        | 23           |
| No Antigen retrieval                | 10         | 4         | 5            |
| Unknown                             | 3          | 0         | 1            |
| **Grand Total**                     | **91**     | **36**    | **29**       |

| HIER modality                      | E-cadherin | β-catenin | p120-catenin |
|-------------------------------------|------------|-----------|--------------|
| Hot plate                           | 20         | 9         | 6            |
| Microwave                           | 7          | 2         | 1            |
| Pressure cooker                     | 4          | 6         | 2            |
| Stainer                             | 28         | 11        | 11           |
| Steamer                             | 2          | 0         | 1            |
| Water bath                          | 3          | 1         | 0            |
| Unknown                             | 11         | 1         | 2            |
| **Other**                           | 1          | 1         | 0            |
| **Grand Total**                     | **76**     | **31**    | **23**       |

| HIER buffer                         | E-cadherin | β-catenin | p120-catenin |
|-------------------------------------|------------|-----------|--------------|
| Acidic                              | 3          | 5         | 2            |
| Low pH (<7,0)                       | 3          | 5         | 2            |
| **Basic pH**                        | **60**     | **24**    | **19**       |
| High pH (>8,0)                      | 38         | 10        | 10           |
| slightly basic pH [7,0-8,0]         | 8          | 6         | 4            |
| Unknown                             | 14         | 8         | 5            |
| **Missing data**                    | **13**     | **2**     | **2**        |
| **Grand Total**                     | **76**     | **31**    | **23**       |
### Supplementary Table 6a: concentration per clone for β-catenin

| Concentration per clone | Number of participants |
|-------------------------|------------------------|
| Clone 14                | 20                     |
| RTU                     | 6                      |
| unknown                 | 5                      |
| 1/100                   | 2                      |
| 1/200                   | 2                      |
| 1/75                    | 1                      |
| 1/150                   | 1                      |
| 1/300                   | 1                      |
| 1.25 µg/ml              | 1                      |
| 1/250                   | 1                      |
| **β-Catenin-1**         | 12                     |
| 1/200                   | 3                      |
| RTU                     | 2                      |
| 1/1000                  | 1                      |
| 1/400                   | 1                      |
| 2.2mg/L                 | 1                      |
| unknown                 | 4                      |
| **17C2**                | 3                      |
| 1/100                   | 1                      |
| RTU                     | 1                      |
| unknown                 | 1                      |
| **CAT-5H10**            | 2                      |
| 1/200                   | 1                      |
| 1/100                   | 1                      |
| unknown                 | 2                      |
| **Grand Total**         | 38                     |

### Supplementary Table 6b: concentration per clone for p120-catenin

| Concentration per clone | Number of participants |
|-------------------------|------------------------|
| Clone 98                | 14                     |
| unknown                 | 5                      |
| RTU                     | 3                      |
| 1/500                   | 1                      |
| 1/200                   | 1                      |
| 1/150                   | 1                      |
| 0.076µg/mL              | 1                      |
| 1/250                   | 1                      |
| 1/300                   | 1                      |
| **EP66**                | 7                      |
| RTU                     | 3                      |
Supplementary Table 6c: concentration per clone for other reported antibodies

| Concentration per clone | Number of participants |
|-------------------------|------------------------|
| Catenin Delta-1 (D7S2M) | 1                      |
| 0.7 µg/mL               | 1                      |
| Cytokeratin 34βE12      | 5                      |
| 1/100                   | 1                      |
| 1/300                   | 1                      |
| 1/50                    | 2                      |
| RTU                     | 1                      |
| P-cadherin (clone 56)   | 1                      |
| 1/100                   | 1                      |
| Grand Total             | 29                     |