# Vaccine Carrier Cold Life and Vial Freezing

Laboratory Testing Report

PATH

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# Introduction

Water-packs used in vaccine carriers may be frozen at –25°C or colder. Before using the frozen water-pack in a vaccine carrier, the water-pack must be conditioned at ambient temperature until the region of ice adjacent to the internal wall of the water-pack has melted and the ice mass in the water-pack becomes mobile within the water-pack. The conditioning step is meant to warm the water-pack to 0°C to eliminate the risk of freezing the vaccine in the vials placed into the carrier. If performed correctly, the conditioning step is effective at preventing vaccine freezing. If the condition step is not performed or is performed inadequately, water-packs with temperatures well below 0°C may be placed into a vaccine carrier with vaccines creating the risk of freezing.

The possibility of health care workers using partially frozen water-packs also exists. Water-packs freeze primarily from the outside to the inside, so a partially frozen water-pack may appear completely frozen. In a freezer containing both partially frozen and completely frozen water-packs, health care worker may accidentally choose a partially frozen water-pack instead of a fully frozen water-pack. When partially frozen water-packs are used, the length of time that the vaccine carrier can maintain vaccines in the 0°C to +10°C temperature range specified by WHO is decreased.

PATH measured the impact of the temperature and degree of freezing of water-packs on vaccine carrier cold life. The vaccine carriers were tested in a test chamber set at +43°C using water-packs cooled to +5°C, and completely frozen at –10°C, and –25°C. Cold life was also tested with water-packs cooled at –30°C in a freezer for 2, 4, and 6 hours to obtain water-packs with differing degrees of partial freezing. These partially frozen water-packs were used to simulate cases in which a health care worker places completely thawed water-packs into a freezer, but then accidentally removes the water-pack for use in a vaccine carrier before the water-pack has completely frozen. In addition to the cold life measurements, the experiments also provided data on the likelihood of vaccines freezing in vaccine carriers when frozen or partially frozen water-packs are used. During the experiments, a simulated vaccine vial containing water was positioned on the bottom of each carrier, centered within the carrier. Centered vials in carriers using –10°C water-packs did not freeze, so additional experiments were performed to assess the freezing of vials in direct contact with –10°C frozen water-packs.

In this work, we measured cold life differently than is specified in the World Health Organization (WHO) Performance, Quality, and Safety (PQS) verification protocol for vaccine carriers. The WHO PQS definition for cold life for vaccine carriers is the length of time from the moment when the container lid is closed until the temperature of the warmest point in the vaccine storage compartment first reaches +10°C (after initially cooling to below +10°C during cooldown), at a constant ambient test temperature of +43°C.[1] The PQS verification protocol to measure cold life uses partially filled water-packs for a simulated vaccine load, three different temperature measurement locations, and temperature probes embedded in metal masses.[2] Because of the differences in measurement technique, the cold life measurements in these tests may be different from what would be measured using the PQS verification protocol. The simplified temperature measurement with a single vaccine vial with a thermocouple in the center of the vial water mass was used to simulate a worst-case scenario in terms of potential vaccine vial freezing by removing additional vaccine load that would help buffer temperature changes in the carrier. Placing the thermocouple in the vial water mass improved detection of vial freezing over what would have been possible using a thermocouple installed in a metal mass positioned adjacent to water-filled vials.

# Testing

## Vaccine carrier cold life and vial freezing

### Materials and methods

To measure the cold life of vaccines in carriers using water-packs cooled to different temperatures and for varying lengths of time, three 2.5-L-capacity vaccine carriers (AOV International, AVC-46) were placed in an environmental chamber at +43°C and 65% relative humidity for 24 hours. Twelve 0.6-L water-packs were filled with tap water to the maximum fill line. The water-packs were then completely frozen at –25°C or –10°C, cooled at +5°C, or were kept at –30°C for 2, 4, or 6 hours to achieve different degrees of freezing. Three 10-mL glass vials were filled with 5 mL of tap water. The glass vials were cooled at +5°C overnight.

To monitor temperatures within the vials and water-packs, all were fitted with thin-wire, type-T thermocouples (Omega, OSK2K3671-13), which were monitored using National Instruments (NI) data acquisition units (NI 9211 with chassis cDAQ-9172) and NI SignalExpress software. For the water-packs, the thermocouple entered through the water-pack closure and the thermocouple tip was positioned approximately halfway into the water-pack vertically between the water-pack vial indentation and the sidewall. For the vaccine vials, the thermocouple was threaded between the vial and the stopper with the thermocouple tip positioned near the center of the volume of the water in the vial. A 1-cm-thick piece of closed-cell foam with a hole cut in the center was used to center the vial on the bottom of each carrier, as shown in Figure 1. A thermocouple was also positioned between the vial and the frozen water-pack to measure the carrier temperature.

Figure 1. Vial centered at the bottom of vaccine carrier for cold life experiments.

Photo: PATH/Dan Myers.

During testing, the cooled water-packs and vials were placed into the warmed carriers, the carriers were closed, and the temperature logging was started. Temperatures inside the water-packs, vials, carrier, and environmental chamber were recorded every 30 seconds until the temperature inside the vial had risen above +10°C. The cold life was calculated as the length of time for the temperature inside the vial to rise above +10°C from the moment the carrier lid was closed.

## Results

The cold life for the three carriers for each water-pack temperature condition are shown in Table 1.

Table 1. Cold life for vaccine carriers.

| Water-pack preparation | Vaccine carrier cold life (h) | | | | |
|---|---|---|---|---|---|
| | Carrier 1 | Carrier 2 | Carrier 3 | Average | Standard deviation |
| Frozen completely at –25°C | 46.5 | 48.3 | 47.3 | 47.3 | 0.9 |
| Frozen completely at –10°C | 41.8 | 42.9 | 44.1 | 42.9 | 1.2 |
| Partial freezing for 2 hours at –30°C | 4.2 | 5.8 | 3.6 | 4.5 | 1.1 |
| Partial freezing for 4 hours at –30°C | 8.5 | 8.8 | 6.4 | 7.9 | 1.3 |
| Partial freezing for 6 hours at –30°C | 27.3 | 25.2 | 23.4 | 25.3 | 2.0 |
| Cooled to 5°C | 0.1 | 0.1 | 0.3 | 0.2 | 0.1 |

The PQS requirements for cold life for long-range vaccine carriers such as the carriers used in these tests is 30 hours. Water-packs frozen at –10°C and –25°C greatly exceeded this cold life. None of the water-packs cooled for 2, 4, or 6 hours at –30°C yielded a cold life that met the requirement. These results suggest that water-packs must be completely frozen to achieve a cold life of 30 hours in long-range vaccine carriers.

The PQS vaccine carrier specifications define cool life using water-packs cooled to +5°C as the length of time required for the warmest temperature in the carrier to rise above +20°C after the lid of the carrier is closed.[2] The PQS specification does not define a required cool life for vaccine carriers. In these tests, the temperature measurements were stopped before the vial temperature reached +20°C because the tests focused on the time the vial spent below +10°C. Extrapolating from the data collected, each carrier had a cool life well in excess of 5 hours. As Table 1 shows, however, most of this time is spent above 10°C, with an average time spent below 10°C of only 12 minutes.

Examples of the temperatures recorded during the course of a cold life test for –10°C, –25°C, and 6 hours at –30°C frozen water-packs are shown in Figures 2, 3, 4, and 5. Figures 2 and 3 for the –25°C frozen water-packs suggest that the water in the vial froze; water in the vial super cooled to –10°C, then quickly froze, raising the temperature briefly to 0°C before the temperature of the ice in the vial dropped back below 0°C. This temperature behavior in the vial was seen in two out of three carriers using frozen –25°C water-packs, suggesting that vials in carriers using –25°C frozen water-packs are at risk of freezing even when not in contact with the frozen water-packs. In Figures 4 and 5 where –10°C frozen water-packs and water-packs partially frozen at –30°C for 6 hours were used, respectively, the temperatures in the vial never dropped to 0°C, indicating that the water never froze.

Figure 2. Temperatures recorded during –25°C frozen water-pack cold life test using carrier 2.

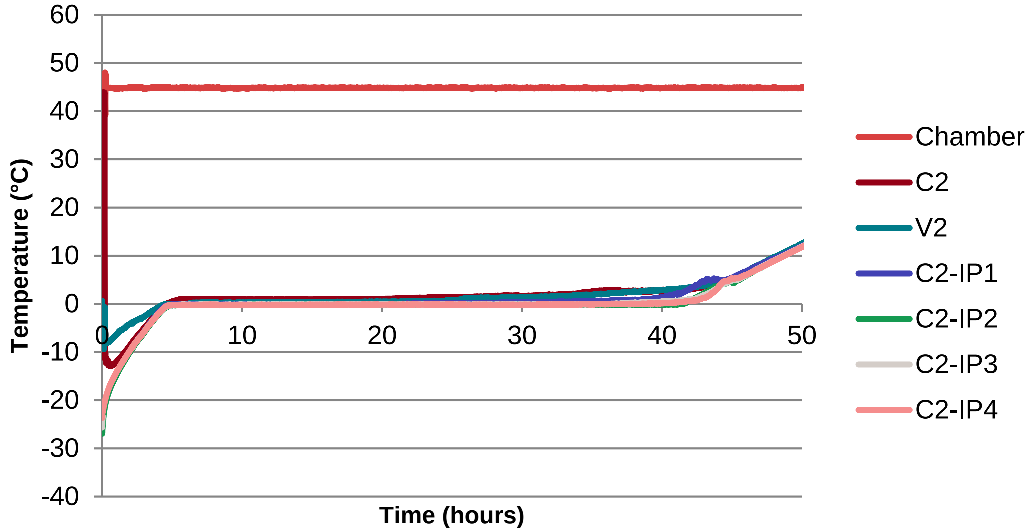
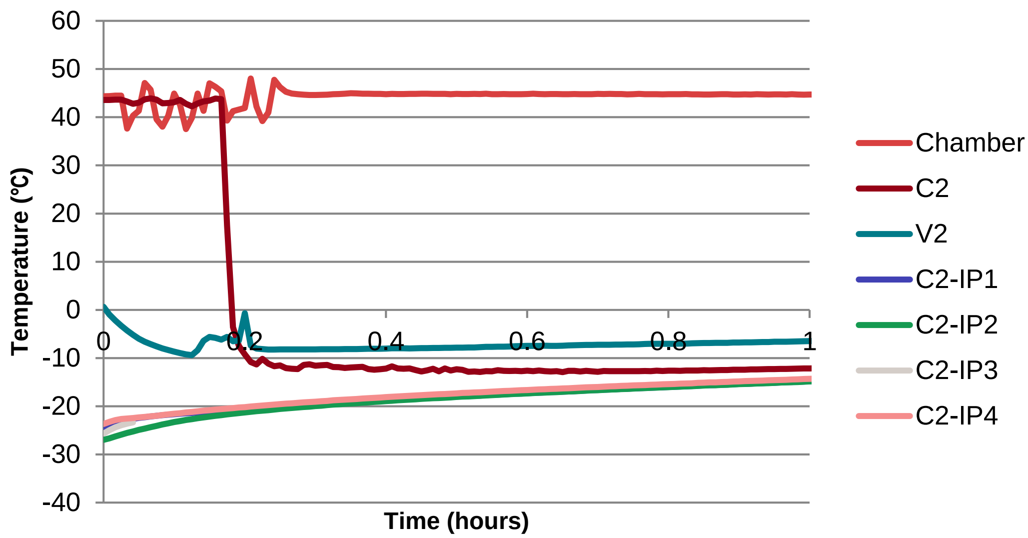
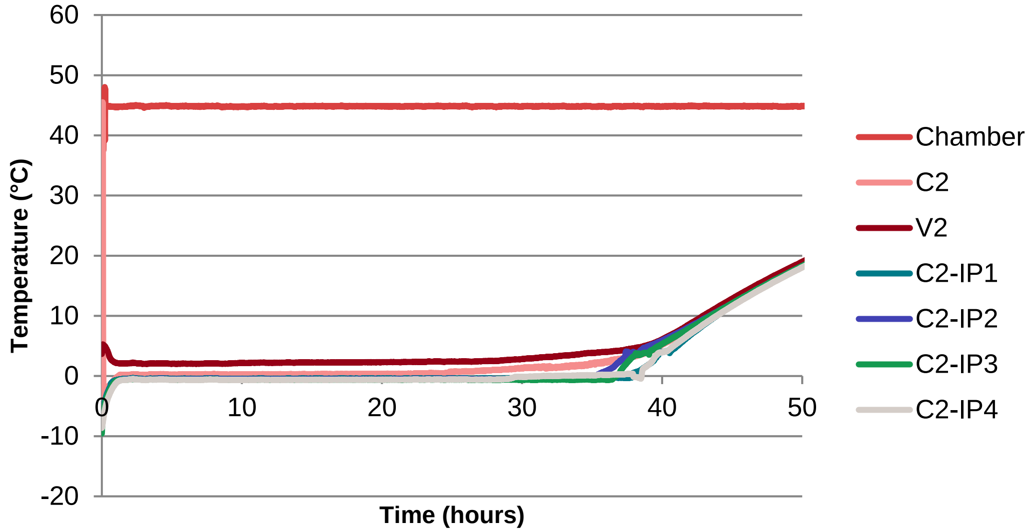



Figure 3. Temperatures recorded during frozen –25°C water-pack cold life test using carrier 2 (detail).



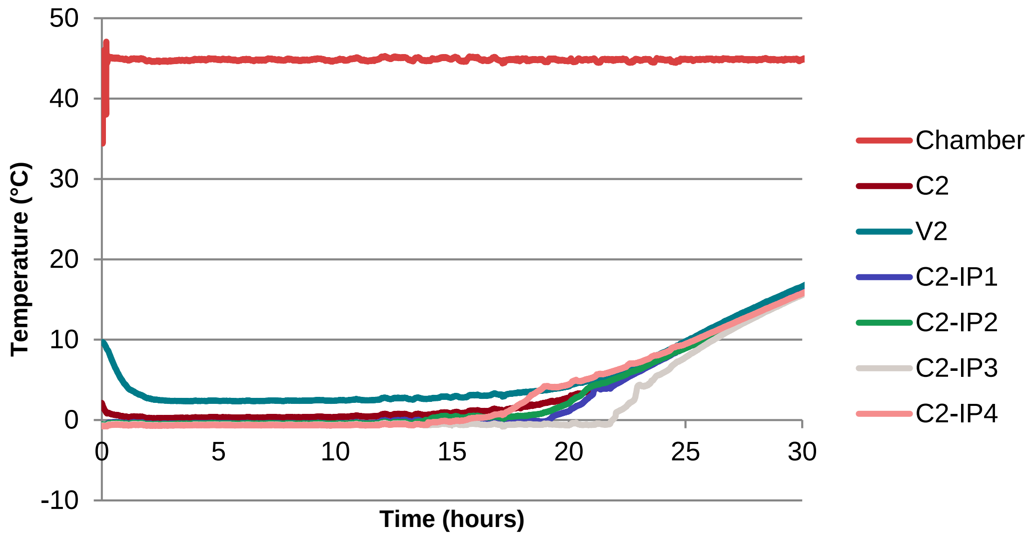

Abbreviations: Chamber, environmental chamber; C2, carrier 2; V2, vial in carrier 2; C2-IP#, carrier 2 water-pack number (1, 2, 3, or 4).

Figure 4. Temperatures recorded during –10°C frozen water-pack cold life test using carrier 2.




Abbreviations: Chamber, environmental chamber; C2, carrier 2; V2, vial in carrier 2; C2-IP#, carrier 2 water-pack number (1, 2, 3, or 4).

Figure 5. Temperatures recorded during 6-hour conditioning at –30°C water-pack cold life test using carrier 2.




Abbreviations: Chamber, environmental chamber; C2, carrier 2; V2, vial in carrier 2; C2-IP#, carrier 2 water-pack number (1, 2, 3, or 4).

The absence of freezing in the vial in all of the carriers with the –10°C frozen water-packs raised the question as to whether all locations within the carrier were free of freezing risk. To further address this question, the cold-life test of the –10°C frozen water-packs was repeated using vials placed at the center of the carrier and in the corner formed by two adjacent frozen water-packs.

## Impact of vial location on freezing risk

### Materials and methods

Six vaccine carriers (Nilkamal, BCVC 44-A) were warmed in an environmental chamber at +43°C and 65% relative humidity for 24 hours. Twenty-four 0.6-L water-packs were filled to the maximum fill mark with tap water. Twelve were held at –10°C for approximately 60 hours and 12 were held at –25°C for approximately 60 hours. All water-packs were completely frozen before use. Twelve 10-mL glass vials were filled with 5 mL of tap water, capped, placed in custom foam holders, and cooled to +5°C overnight. Vials and water-packs were fitted with type-T thermocouples, the same as for the carrier cold life tests, and monitored using the same data acquisition software and hardware. Thermocouple tip locations in the water-packs and vials were the same as for the cold life tests. A thermocouple was placed between the two vials about 2 cm above the surface of the foam holder to measure the temperature of the air in the carrier near the vials. Vials were positioned inside the carrier approximately halfway up the frozen water-packs with one vial in the corner formed by two water-packs and the other in the center, as shown in Figure 6. The corner vial was touching both frozen water-packs. After closing the carriers and the environmental chamber, temperatures of the water-packs, vials, carriers, and environmental chamber were recorded every 30 seconds until the vial temperatures reached 10°C. The entire experiment was repeated three times.

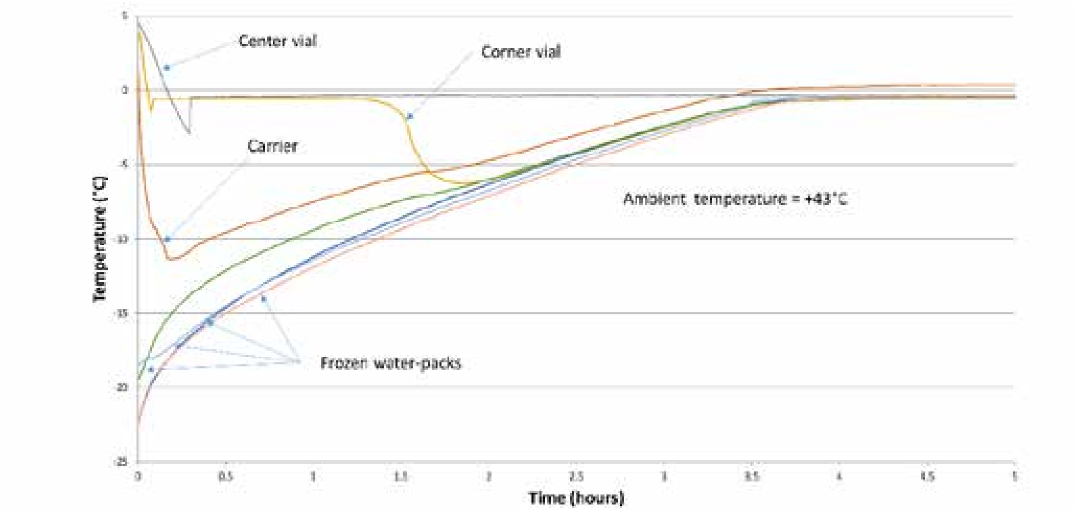
Figure 6. Position of vials in carrier with frozen water-packs for freezing tests.

Photo: PATH/Dan Myers.

### Results

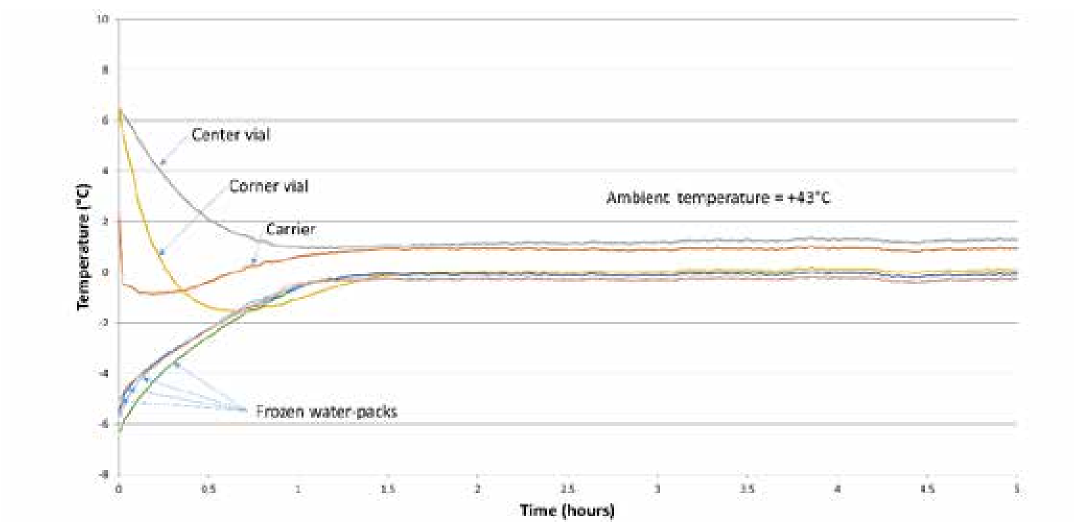
Performing the cold life test in triplicate with three carriers at each frozen water-pack temperature yielded nine data sets for each water-pack freezing temperature. The temperature profiles for the vials, carriers, and water-packs of a given water-pack temperature were very similar. Representative temperature profiles are given in Figures 7 and 8 for the –25°C and –10°C water-packs, respectively.

Figure 7. Vial freezing experiment using –25°C frozen water-packs.




For carriers with the –25°C frozen water-packs, both the center and corner vials cooled to temperatures at or below 0°C. In eight out of nine repetitions of the tests, the corner vial cooled below 0°C, then suddenly warmed to 0°C, indicating that the supercooled water in the corner vial had frozen. In six out of nine tests, the center vial exhibited the same supercooling and freezing behavior as the majority of corner vials. In the other three tests, the center vial cooled below 0°C reaching the water-pack temperatures, then warmed with the water-packs. In these three cases, it is uncertain whether the center vial ever froze even though the temperature in the vial dropped well below 0°C.

Figure 8. Vial freezing experiment using –10°C frozen water-packs.




For carriers with the –10°C frozen water-packs, the corner vial always cooled to temperatures at or below 0°C. During the first two repetitions of the test, the center vial cooled to between 0°C and 2°C. The vials in these tests, however, were between 6°C and 8°C when they were placed into the carriers due to warming that took place during transfer of the vials from the conditioning refrigerator to the carriers. When the vials were cooled to slightly below 5°C during the third repetition so that their starting temperature in the carrier would be closer to 5°C, the center vial would cool to 0°C during the experiment.

# Conclusions

Cold life testing of 2.5-L long-range vaccine carriers show that to reach the required cold life of 30 hours, water-packs must be completely frozen. Vials in carriers using water-packs completely frozen at –10°C and –25°C had cold lives in excess of 40 hours. Vials in carriers using water-packs cooled for 2, 4, or 6 hours at –30°C to achieve partial freezing did not have cold lives that met the 30-hour threshold. These results do not imply that the current frozen water-pack conditioning practice results in partially frozen water packs that would reduce carrier holdover below 30 hours. Conditioning frozen water-packs until the ice mass in the water-pack just becomes mobile leaves a larger mass of ice in the water-pack than is formed in the partially frozen water-packs tested in this work.

The time spent below 10°C for vials placed in carriers using cool-packs was very short, on average only 12 minutes. This result suggests that cool-packs can only be used for preservation of vaccine vials for very short periods of time.

The vial freezing tests confirm that freezing of vaccine vial contents is a concern for carriers using water-packs completely frozen at –10°C and –25°C and used without conditioning. Vials will reach freezing temperatures for both frozen water-pack temperatures if the vials are located in the corner formed by two water-packs and are touching the water-packs. Vials located in the center of the carrier exhibit variable temperature behavior. For center vials in carriers with –25°C frozen water-packs, the vials cool to 0°C and appear to freeze in most cases. For center vials in carriers with -10°C frozen water-packs, internal vial temperatures may reach 0°C if the vials enter the vaccine carrier at 5°C. However, in actual use, as in tests with vials cooled to 5°C, vials centered in the carrier may not cool to 0°C or below dependent on warming of the vials during transfer from the vaccine refrigerator to the carrier.

# References

1. World Health Organization (WHO). *Vaccine carrier*. *Performance, Quality and Safety (PQS) independent type-testing protocol E004/VC01-VP.2*. Geneva: WHO; 2008. https://apps.who.int/immunization_standards/vaccine_quality/pqs_catalogue/catdocumentation.aspx?id_cat=18.

2. World Health Organization (WHO). *Vaccine carrier*. *Performance, Quality and Safety (PQS) performance specification E004/VC01.2*. Geneva: WHO; 2008. https://apps.who.int/immunization_standards/vaccine_quality/pqs_catalogue/catdocumentation.aspx?id_cat=18.